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(54) **Processes for preparation of aloe products, products produced thereby and compositions thereof.**

Several related processes are disclosed for extracting the active chemical substance in the aloe plant from a leaf of the aloe plant, as are compositions based on ethanol precipitation products of the juice of the aloe plant with the precipitation product being substantially non-degradable and lyophilized. Furthermore, the application relates to a base formulation for the production of aloe vera-containing topical products and to moisture resistant emulsion formulations based on aloe juice. The application also describes aloe-based counter-irritant formulations based on aloe juice.

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PROCESSES FOR PREPARATION OF ALOE PRODUCTS, PRODUCTS PRODUCED THEREBY AND COMPOSITIONS THEREOF

BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention pertains to the field of processing aloe plants and removing portions of said plant for processing same into compositions for topical and internal applications, and compositions of matter comprising said portions of aloe.

2. Description of the Prior Art, and Other Information

Aloe vera is not a cactus plant, as widely believed, but rather a member of the lily family. Aloe is a tropical or subtropical plant characterized by lance-shaped leaves with jagged edges and sharp points. There are about 360 species of aloe plants known. Harding, Aloes of the World: A Checklist, Index and Code, Excelsa 9: 57 - 94 (1979). They seem to thrive in hot, arid areas and are widely scattered from the Mediterranean Sea, Middle East, Africa, China, Japan, Mexico and the southern U.S.A. A few of the important species used for their medicinal properties are *Aloe barbadensis* Miller (aloe vera), *A. arborescens*, *A. plicatilis*, *A. vahombe*, *A. saponaria*, *A. africana*, *A. ferox* and *Aloe perryi*. Reynolds, Aloes of Tropical Africa and Madagascar, The Trustees, The Aloe Book Fund, Mbabane Swaziland. However, *A. barbadensis* Miller is generally recognized as the "true aloe" because of its wide use and, reportedly, most effective healing power, although in Japan, *A. arborescens* Miller traditionally has been used as a folk remedy for various ailments ranging from gastrointestinal disorders to athlete's foot.

Aloe vera is a perennial plant with turgid green leaves joined at the stem in a rosette pattern. The leaves of a mature plant may be more than 25 inches long with saw-like spikes along their margins.

Aloe barbadensis is native to northern Africa, and was introduced into the island of Barbados about 1630. A variety of

Aloe barbadensis (called Aloe chinensis Baker) was introduced by William Anderson into Curacao in 1817 from China. It was cultivated in Barbados for its laxative fraction until the middle of the nineteenth century, when the industry began to wane. Curacao aloe, which is often called Barbados aloe, comes from the Dutch islands of Aruba and Bonaire. The market for laxative aloe diminished as better and safer laxatives were developed.

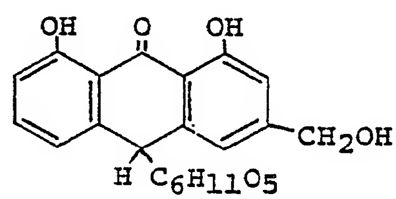
The plant contains two separate juice materials. One is made from the clear cellular gel and the second, a yellow juice, is contained in the pericyclic cells of the vascular bundles located at the junction between the rind (cortex) and the internal fillet (Figures 1 and 2 - 1 is the clear cellular gel, 2 the yellow juice containing anthraquinones, 3 is the rind).

Aloe vera contains two major liquid sources a yellow latex (exudate) and the clear gel (mucilage). The dried exudate of Aloe barbadensis Miller leaves is referred to aloe. The commercial name is Curacao aloe. It is composed mainly of aloin, aloe-emodin and phenols. Bruce, South African Medical Journal, 41: 984 (1967); Morrow et al., Archives of Dermatology, 116: 1064-1065 (1980); Salek et al., Corrosion Prevention & Control, 9-10 (1983); Mapp et al., Planta Medica, 18: 361-365 (1970); Ranwald, Archives Pharmazie, 315: 477-478 (1982). A number of phenolics, including anthraquinones and their glycosides, are known to be pharmaceutically active. Bruce, Excelsa 5: 57-68 (1975); Suga et al., Cosmetics and Toiletries, 98: 105-108 (1983).

The mucilaginous jelly from the parenchymal cells of the plant is referred to as Aloe vera gel. There are generally no anthraquinones to decompose and cause discoloration of the gel unless the gel is contaminated by an improper processing technique.

Aloe vera gel is about 98.5% water by weight. More than 60% of the total solid is made up of polysaccharides of carbohydrate origin. Organic acids and inorganic compounds, especially calcium oxalate, account for the remainder of the solid.

For centuries the yellow juice has been dried and used as a laxative. For example, in the Dutch islands of Aruba and Bonaire, leaves are cut in March and April, then placed cut end downward on a V-shaped trough which is inclined so that the latex can be fed into a cooking vessel. Varro E. Tyler, PHARMACOGNOSY at pp. 60-63 (Lea and Febiger, Philadelphia, 1981). The dried latex of the leaves of Aloe barbadensis Miller or other Aloes ranges in color from reddish-black to brownish-black to dark brown in color. The taste of each variety of dried latex is nauseating and bitter; also the odor is characteristic and disagreeable. It contains a number of anthraquinone glycosides; the principal one is called barbaloin (aloe-emodin anthrone C-10 glucoside). This dried latex, that has been sold for centuries as a laxative, is commonly called aloe or aloes.



(±) Barbaloin

The active laxative constituents vary quantitatively and qualitatively according to the species and environmental growing conditions. For example, Curacao aloe contains two and one half times as much aloe-emodin when compared to Cape Aloe, and Curacao aloe contains an appreciable amount of free chrysophanic acid not present in other types of aloe (Tyler, op. cit.). Many companies sell Aloe products that contain a large amount of the yellow sap even claiming them to be beneficial. Both juices become mixed together by the juice extraction process which is used by many producers.

The following species of Aloe have been used commer-

cially for their yellow sap, which was dried and used as a laxative. Arthur Osol et al., THE UNITED STATES DISPENSATORY AND PHYSICIANS' PHARMACOLOGY, (J. B. Lippencott Co., Philadelphia, 1980) at pp. 42-43:

"1. Aloe Perryi Baker. - The true Socotrine aloe is a perennial herb, growing abundantly on the island of Socotra especially in the limestone tracts, from the sea level to an altitude of 3,000 feet and also found in eastern Africa and in Arabia. It has a trunk one foot high which bears on its summit a dense rosette of pale green or reddish, suc culent, lanceolate leaves with brown-tipped marginal spines.

"2. Aloe barbadensis Mill. - (A. vera "L"; A. vulgaris Lamarck). - This species, which is the source of Curacao aloe, has a very short, woody stem, and lanceolate embracing leaves, of glaucous green color, with hard, pale spi nes. It has bright yellow flowers arranged in a spicate inflorescence. A. barbadensis is a native of southeastern Europe, northern Africa, and Madagascar. It is cultivated in Italy, Sicily, Malta, and especially in the West Indies.

"3. Aloe ferox Miller, one of the three South African, tree-like species yielding Cape aloe, is one of the tallest species of the genius. [sic] It has a forked stem 5 to 15 feet long, 4 to 6 inches in diameter; furnished at the top with a dense rosette containing 30 to 50 lanceolate leaves 1.5 to 2 feet long, with prickles.

"4. Aloe africana Mill., an aborescent South African species, has a simple tall trunk which bears on its summit a few triangular-oblong,

glaucous, green leaves with large, horny marginal teeth. It is a native of the Cape Colony.

"5. Aloe spicata Baker. (A. Eru var. cornuta Berger) is a tall, branched aloe indigenous to tropical southern Africa. It possesses pale, glossy, fleshy leaves with white blotches and a panicle of campanulate yellow flowers."

Aloe as a laxative is best summarized by the text of Goodman and Gilman, as follows:

"The yellow sap has not been subjected to controlled clinical comparison with the other anthraquinones but has the reputation of being the most irritating of these cathartics. It produces considerable griping and pelvic congestion, and excessive doses may cause nephritis. It is still described in the U.S.P., but only for pharmaceutical reasons. Both aloe and aloin, a mixture of active glycosides, should be abandoned."

Goodman and Gilman, Eds., THE PHARMACOLOGICAL BASIS OF THERAPEUTICS, at 984, (MacMillan Publishing Co., Inc. New York 1975.)

Whole leaves, exudates and fresh gels of Aloe plants have been used for a variety of human afflictions. Evidence of their use as a medicinal remedy can be traced to the Egyptians of 400 BC. Aloe vera was also used to embalm the dead, as well as to protect the embalmers from the death-causing agent. Other early civilizations used aloe vera for skin care, to relieve insect stings and bites, to treat scratches and ulcerated skin, to promote wound healing, to prevent hair loss and as a purgative. It was the traditional medicine of many cultures as an anthelmintic, cathartic and stomachic and was used inter alia for leprosy, burns and allergic conditions. Cole et al., Archives of Dermatology and Syphilology, 47: 250 (1943); Chopra et al., Glossary of Indian Medicinal Plants, Council of Scientific and

Industrial Research, New Delhi; Ship, Journal of the American Medical Association, 238: 1770-1772 (1977); Morton, Atlas of Medicinal Plants of Middle American Bahamas to Yucatan, Charles C. Thomas Publisher, 78-80 (1981); Diez-Martinez, La Zabila, Communicado NO. 46 Sobre Recursos Bioticos Potenciales del Pais, INIREB, Mexico (1981); Dastur, Medicinal Plants of India and Pakistan; D.B. Taraporevala Sons & Co., Private Ltd., Bombay 16-17 (1962).

Aloe vera has enjoyed a long history of lay acceptance as possessing "curative" or "healing" qualities. Over the last few years, numerous books and articles meeting scientific standards have been written on Aloe vera. Organizations such as the Aloe Vera Council and recognized medical institutions, through publications and case-histories of physicians, veterinarians and other scientists, have given credence to the "aloe phenomenon". Aloe vera has been featured extensively in the field of dermatology, especially for treating radiation-caused skin conditions. Mackee, X-Rays and Radium in the Treatment of Diseases of the Skin, 3rd Ed., Lea and Febiger, Philadelphia, 319-320 (1938); Rovalti et al., Industrial Medicine and Surgery, 28: 364-368 (1959); Zawahry et al., Quotations From Medical Journals on Aloe Research, Ed. Max B. Skousen, Aloe Vera Research Institute, Cypress, California, 18-23 (1977); Cera et al., Journal of the American Animal Hospital Association, 18: 633-638 (1982). The body of scientific literature documenting medical applications in digestive problems, as a virucidal, bactericidal and fungicidal agent and in gynecological conditions is extensive and has been adequately reviewed by Grindley and Reynolds (Journal of Ethnopharmacology, 16: 117-151 (1986)).

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The importance of chemicals found in aloes is indicated by the fact that they have been listed in every known national pharmacopeia. U.S. Pharmacopeia, 20th Revision, The National Formulary, 14th Edition, United States Pharmacopeial Convention, Inc., Rockville, Maryland, July 1, 1980. However, the U.S. Pharmacopeia describes the yellow sap drug portion of aloes but not the mucilage. The fresh unpreserved gel is about 98.5-99.2% water. The total solid that remains after the water has been removed ranges from 0.8 to 1.5%. The mucilage, sugars, fiber, proteins, ash, fats, aloin and resin are the major constituents of the solid. Robson et al., Journal of Burn Care Rehabilitation, 3: 157-163 (1982). Compositions that include enzymes, organic acids, inorganic salts, amino acids and alkaloids have been noted. Rowe et al., Journal of the American Pharmaceutical Association, 30: 262-266 (1941); Roboz et al., Journal of the American Chemical Society, 70: 3248-3249 (1948); Waller et al., Proceedings of Oklahoma Academy of Science, 58: 69-76 (1978). Depending on the way in which the leaves are processed, mucilage and sugars are the major components of the dehydrated gel. The sugars found are galactose, glucose, mannose, rhamnose, xylose and uronic acids. Although conflicting reports have been observed, the mucilage is mainly composed of

mannan or glucomannan. Eberendu et al., The Chemical Characterization of Carrisyn™ (in preparation); Mandal et al., Carbohydrate Research, 87: 249-256 (1980b); Roboz et al., Journal of the American Chemical Society, 70: 3248-3249 (1948); Gowda et al., Carbohydrate Research, 72: 201-205 (1979); Segal et al., Lloydia, 31: 423 (1968).

It is therefore evident that for centuries, the aloe plant has been considered to have, and has been used for its medicinal and therapeutic properties without any clear understanding or scientific analysis of the bases for such properties. Note U.S. Patents U.S.-A-3,892,853 to Cobble and U.S.-A-4,178,372 to Coats, both of which teach a process for producing an alleged stabilized (i.e. bacteriologically stable) aloe juice by extracting the mucilage from the aloe leaves and adding a mild oxidant (H_2O_2). No reference is made in these patents to removing yellow sap, or to adverse properties in aloe juice due to the presence of yellow sap. In fact, both the '853 and '372 patents attribute adverse properties prior to processing to beta "and perhaps" alpha globulin proteins (col. 3, lines 41-43 of the '853; col. 3, lines 42-47 of the '372). Furthermore, attempts to produce various commercial products from extracts and derivatives of the aloe plant have met with varying degrees of success and failure.

Prior to this work, the controversy over the identity of the active substance(s) in Aloe vera had not been settled. It is therefore important to clearly distinguish between the components present in the gel and those found in the exudates. A larger part of the gel is a mucilage of mainly polysaccharide nature with minor amounts of various other compounds. It has been observed that in some of the activities observed there may be some synergistic action between the polysaccharide base and other components. Leung, Excelsa 8: 65-68 (1978); Henry, Cosmetics and Toiletries, 94: 42-50 (1979). For example, several workers report that the effective components for wound healing may be tannic acid (Freytag, Pharmazie, 9: 705 (1954)) and a kind of polysaccharide. Kawashima et al., Chemical Abstracts, 93: 13075

(1979). Mackee, supra, noted that the gel, not the rind or the exudate, was responsible for the beneficial effects in the treatment of radiation burns, and he stressed the importance of using fresh leaves for effective treatment. Polysaccharides degrade with time, and certain molecular weight sizes may be necessary to elicit a specified pharmacological response. Goto et al., Gann, 63: 371-374 (1972).

There are many examples in the literature that polysaccharides can exhibit pharmacological and physiological activities without help from other components. G. Gialdroni-Grassi, International Archives of Allergy and Applied Immunology, 76 (Suppl. 1): 119-127 (1985); Ohno et al., Chemical and Pharmaceutical Bulletin, 33: 2564-2568 (1985); Leibovici et al., Chemico-Biological Interactions, 60: 191-200 (1986); Ukai et al., Chemical and Pharmaceutical Bulletin, 31: 741-744 (1983); Leibovici et al., Anticancer Research, 5: 553-558 (1985). One such example relates to development of atherosclerosis. Hyperlipidemia in the general population and especially in familial hypercholesterolemia is associated with coronary heart disease and death. In countries where dietary fiber intake is high, atherosclerosis appears to be uncommon. Trowell et al., Editors, Refined Carbohydrate Foods and Disease, London, Academic Press, 207 (1975). Pectin and guar are reported to lower cholesterol in normal and hyperlipidemic patients. Kay et al., American Journal of Clinical Nutrition, 30: 171-175 (1977). Locust bean gum, a polysaccharide composed of mannose and galactose, decreased the plasma lipoprotein cholesterol concentrations in both normal and familial hypercholesterolemic subjects. Zavoral et al., American Journal of Clinical Nutrition, 38: 285-294 (1983). Addition of guar gum to carbohydrate meals decreased the postprandial rise of glucose in both normal and diabetic subjects. Jenkins et al., Lancet, 2: 779-780 (1977). Kuhl et al., (Diabetes Care, 6 (2): 152-154 (1983)) demonstrated that guar gum exhibited glycemic control of pregnant insulin-dependent diabetic patients.

The anti-tumor activity of polysaccharides has been

widely reported. Polysaccharides prepared from Lentinus cyathiformis are known to increase hosts' defense against tumors. Rethy et al., Annales Immunologiae Hungaricae, 21: 285-290 (1981). There are several reports that polysaccharides from mushroom, yeast or bacterial extracts can elicit a high degree of host defense activity against viral and tumor infestations. Chihara et al., Nature, 222: 687 (1969); Schwartzman, Proceedings of the Society for Experimental Biology and Medicine, 29: 737 (1932); Rethy, X. International Congress of Microbiology; Moscow, 642 (1966). Suzuki et al. (Journal of Pharmacobio-Dynamics, 7: 492-500 (1984) also reported anti-tumor activity of a polysaccharide fraction (GF-1) extracted from cultured fruiting bodies of a fungus, Grifola frondosa. This fraction showed equivalent, high levels of inhibiting activity when administered intraperitoneally (IP), intravenously (IV) and intratumorally (IT). However, oral administration (PO) was not effective. The GF-1 fraction also exhibited anti-tumor action against the solid form of Meth A fibrosarcoma and MM 46 carcinoma in mice. Lentinan, which is a 6-branched B-1-3-linked glucan similar to GF-1, was ineffective against Meth A fibrosarcoma. Chihara, The antitumor polysaccharide Lentinan: an overview; "Manipulation of Host Defense Mechanisms"; ed. by Aoki et al., Excerpta Medica, North Holland, 1-16 (1981); Sasaki et al., Carbohydrate Research, 47: 99-104 (1976). Synthesized branched polysaccharides were reported to demonstrate activities against tumors. Matsuzaki et al., Makromol. Chem., 186: 449 (1985). Matsuzaki et al. (Makromol. Chem., 187: 325-331 (1986)) synthesized branched polysaccharides, which showed significant activities, from ivory nut mannan (B-(1-4)-D-mannopyranose) and B-(1-4)-linked glucomannan. A partially acetylated linear B-(1-3)-D-mannan extracted from fruit bodies of Dictyophoria indusiata Fisch, also exhibited anti-tumor activity. Hara et al., Carbohydrate Research, 143: 111 (1982). It appears that anti-tumor action depends on the type of polymer main chain and its degree of polymerization, because B-(1-3)-glucan-type polymers show higher anti-tumor activity than B-(1-4)-glucan and

hemicellulosic polymers. Matsuzaki et al., Makromol. Chem., 187: 325-331 (1986). A carboxymethylated derivative of B-(1-3)-glucan obtained from bacterial culture filtrate caused severe cell loss from established sarcoma 180 tumors within 2 hours after the injection of the derivative. Baba et al., Journal of Immunopharmacology, 8: 569-572 (1986). The same author observed a compensatory increase in polymorphonuclear leukocytes due to injection of the substance. Incidentally, bestatin, a dipeptide known to possess immune-modulating and anti-tumor activity (Ishizuka et al., Journal of Antibiotics, 32: 642-652 (1980)), influenced neither the tumor yield nor the polymorphonuclear leukocyte count. Baba et al., supra.

There are numerous reports on the anti-tumor effect of sulfated polysaccharides, including heparin (Jolles et al., Acta Univ. Int. Cancer, 16: 682-685 (1960); Suemasu et al., Gann, 61: 125-130 (1970)), sulfated laminaran and dextran. Jolles et al., British Journal of Cancer, 17: 109-115 (1963). Yamamoto et al. (Japanese Journal of Experimental Medicine, 54: 143-151 (1984)) reported enhancement of anti-tumor activity of a fucoidan fraction by further sulfation. The sulfated product demonstrated activity against L-1210 leukemia. The authors postulated that the mechanism of the anti-tumor action might be due partly to inhibition of invasive growth of L-1210 cells, as a result of electrostatic repulsion between the tumor cell and mesothelial cells. Yamamoto et al., supra. Polysaccharides with sulfate groups are also reported to be human T cell mitogens and murine polyclonal B cell activators. Sugawara et al., Microbiological Immunology, 28: 831-839 (1984). Generally, homopolysaccharides of high molecular weight with sulfate groups possess these properties. Dorries et al., European Journal of Immunology, 4: 230-233 (1974); Sugawara et al., Cell Immunology, 74: 162-171 (1982).

It has been reported that glucan extracted from the yeast Saccharomyces cerbisiae is a modulator of cellular and humoral immunity. Wooles et al., Science, 142: 1078-1080 (1963). The polysaccharide also stimulated proliferation of

murine pluripotent hematopoietic stem cells, granulocyte macrophage colony-forming cells and cells forming myeloid and erythroid colonies. Pospisil et al., Experientia, 38: 1232-1234 (1982); Burgaleta et al., Cancer Research, 37: 1739-1742 (1977). Maisin et al. (Radiation Research, 105: 276-281 (1986)) also reported that IV administration of a polysaccharide induced protection of murine hematopoietic stem cells against x-ray exposure, thereby decreasing the mortality of the mice so exposed.

V. Lackovic et al., (Proceedings of the Society for Experimental Biology and Medicine, 134: 874-879 (1970)) took yeast cell-wall and extracted all constituent matter leaving only "mannans" that he found were responsible for the induction of α-interferon production by monocytes. The "purified mannans" alleged to be responsible for the physiologic response had a molecular weight of 5,500-20,000 Daltons. He theorized the mannans stimulated mouse peritoneal macrophages to produce the α-interferon. He does state that his mannans isolated showed no toxicity and "they are poor antigens". There was no mention by Lackovic et al. of the use of these "purified mannans" for antiviral activity or for IL-1 stimulation. We submit Lackovic et al.'s "purified mannans" comprised an assortment of unknown and unidentified substituted and unsubstituted mannans.

Seljelid et al., (Experimental Cell Research, 131: 121 (1981)) have observed that insoluble or gel-forming glycans activated macrophages in vitro, whereas the corresponding soluble glycans did not. They postulated that the orientation in which the glycan was presented to the mononuclear phagocyte was decisive for activation. Bogwald et al. (Scandinavian Journal of Immunology, 20: 355-360 (1984)) immobilized glycans that had a stimulatory effect on the macrophages in vitro. This led the authors to believe that the spacial arrangement of the glycan was decisive for the effect on the macrophages in vitro. A purified polysaccharide isolated from Candida albicans induced an antibody response by human peripheral

blood lympocytes in vitro. Wirz et al., Clinical Immunology and Immunopathology, 33: 199-209 (1984). There were significant differences between the anti-candida antibodies in sera of normal and candida-infected individuals. Wirz et al., supra.

The anti-viral activity of polysaccharides and polysaccharides linked to peptides has been observed. Suzuki et al., Journal of Antibiotics, 32: 1336-1345 (1979). Suzuki et al., supra, reported an antiviral action of peptidomannan (KS-2) extracted from culture mycelia of Lentinus edodes. Both oral (PO) and intraperitoneal (IP) administration increased the peak serum interferon titer, which protected mice against viral infections. This was different from dextran phosphate (DP-40) (Suzuki et al., Proceedings of the Society For Experimental Biology and Medicine, 149: 1069-1075 (1975)) and 9-methylstreptimidone (9-MS) (Saito et al., Antimier, Agent & Chemotherapy, 10: 14-19 (1976)), which induced higher titers of interferon in mice only if administered intravenously (IV) or intraperitoneally (IP).

Anti-inflammatory activity of Aloe vera gel has been widely reported by both oral testimonies and respected scientific journals. Rubel (Cosmetics and Toiletries, 98: 109-114 (1983)) discussed fully the possible mechanism of the anti-inflammatory effect of aloe gel. Ukai et al., (Journal of Pharmacobio-Dynamics, 6: 983-990 (1983)) noted anti-inflammatory activity of polysaccharides extracted from fruit bodies of several fungi. The polysaccharides demonstrated a significant inhibitory effect on carrageenan-induced edema. One of the polymers, O-acetylated-D-mannan (T-2-HN), in addition demonstrated a more marked inhibitory effect than phenylbutazone on scald hyperalgesia. Ukai et al., supra. The assertion that the polysaccharide is free from protein and lipids strongly suggests that the anti-inflammatory effect is due to the acetylated mannan only. Other researchers have also reported anti-inflammatory effects of complex polysaccharides (Saeki et

al., Japanese Journal of Pharmacology, 24: 109-118 (1974)), glycoproteins (Arita et al., Journal of Pharmacology, 24: 861-869 (1974)) and sulfated polysaccharides (Rocha et al., Biochemical Pharmacology, 18: 1285-1295 (1969)).

Literature reports that polysaccharides possess pharmacological and physiological activities continue to flood the pages of well respected scientific journals. It is therefore not illogical that the mucilaginous gel of the Aloe vera, which is essentially a polysaccharide, holds the secret to Aloe vera's medicinal properties. The discrepancies over whether the polysaccharide is a glucomannan, mannan, pectin or of some other composition are a result of chemical purification steps. By processing aloe according to the present invention, a partially acetylated polymannose has been consistently isolated as the major polysaccharide with pharmacological activity. Yagi et al., (Planta Medica, 31: 17-20 (1977)), using a slightly modified extraction method, isolated acetylated mannan (aloe mannan) from Aloe arborescens Miller var. natalensis. Ovodova (Khim. Prior. Soedin, 83: 93833 (1975)), however, earlier isolated pectin as the main component of the same aloe species.

As discussed above, the biological activity of polysaccharides has been recognized for many years. Polysaccharide materials recovered from plants, yeast and bacteria have demonstrated direct biological activity by eliciting an increase in host defense systems. This reaction is primarily manifested by increased host surveillance for other antigenic substances. Polysaccharides serve as adjuvants (Freund's, etc.) and immunomodulators. They also function as unique T cell-independent antigens. Both cellular and humoral immunity may be affected, and increased phagocytosis of infectious organisms and tumor cells has been observed, as has enhanced production of immunoglobulins.

The structure of these immunologically active polysaccharides and the types of structural variations appear to be the factors that control their potency and toxicity.

Their mode(s) of action remain poorly understood; however, recent evidence indicates that several polysaccharides induce lymphocytes and macrophages to produce a wide range of immunologically active substances. The composition of the present invention possesses all of the attributes of these immunologically active substances; it is among the most potent of all known biologically active polysaccharides but differs in that no toxicity has been observed. It also manifests specific antiviral activity through alteration of viral glycoprotein synthesis.

THE PROBLEMS TO WHICH THE INVENTION IS ADDRESSED

Because of this lack of knowledge about the aloe plant and its characteristics, previous methods employed for the processing of the plant and its components resulted in end products which did not consistently achieve desired results because of the presence of yellow sap, which was known to have a laxative effect, Goodman and Gilman, op cit. For example, conventional state of the art processes for the production of various aloe products typically involve crushing (pressure rollers), grinding (e.g., use of Thompson aloe leaf slitter), or pressing (TCX pressure extruder) of the entire leaf of the aloe plant to produce an aloe vera juice, followed by various steps of filtration and stabilization of the juice. The resulting solution was then incorporated in, or mixed with, other solutions or agents to produce the desired end use product which could be, for example, a cosmetic, a health food drink, or a topical ointment.

The principal disadvantage of such state of the art processes is the failure to recognize, and to take into account, that different components of the aloe leaf have characteristics that may not only be inconsistent with the intended use of the final product, but in many instances are deleterious to such use. For example, research conducted incident to the process of the present invention revealed that certain components of the aloe leaf produce cytotoxic activity, U. S. Pat. 3,892,853, while other components

stimulate cell growth. In other instances it was discovered that components of the aloe leaf which are advantageously used in the production of a skin ointment can actually be harmful if used in the ~~the~~ production of a drink to be ingested. Winters et al. concluded that "The Cytotoxic effects of commercially prepared aloe vera gel fractions on normal human and tumor cells in culture suggest that these commercial preparations contain substances introduced during commercial processing which can alter the levels of lectin-like activities and can markedly disrupt the in vitro attachment and growth of human cells". Id at 95. Winters et al. reported to the instant applicant that he used material received from Coats as a commercial stabilized aloe preparation.

Winters et al. found that freshly extracted aloe juice was beneficial to growth of human skin (fibroblast) cells while state-of-the art commercial aloe preparations were actually toxic: "In contrast, fractions of 'stabilized' aloe vera gel were equally cytotoxic for human normal and tumor cells in vitro". W. D. Winters et al., "Effects of aloe Extract on Human Normal and Tumor Cells In Vitro", ECO. BOTANY 35(1) at 89-95 (1981).

By using prior art processes which produced a juice or extract from essentially the entire leaf of the aloe vera plant (or at least a substantial portion thereof), containing an adulterated mixture or conglomeration of the different characteristic portions of such leaf, the resulting product necessarily included components which, while useful for certain end products, actually constituted contamination for certain other end uses. Even if the contamination that was present did not constitute a hazard, at the very least the resulting juice constituted a diluted mixture of components, rather than a concentrate of desired properties for certain other end uses.

More recent studies indicate that the yellow sap is also toxic to human skin cells. Ivan E. Danhof et al., "Stabilized Aloe Vera: Effect on Human Skin Cells", DRUG AND COSMETIC IND. at

52-54, 105-106 (August, 1983) (not admitted to be prior art to the present invention) and should be minimized in topical products where it can come in contact with broken or damaged skin. Idiosyncratic hypersensitivity has been demonstrated in processed Aloe vera gel that consists of a variable mixture of yellow sap. David M. Morrow, M.D., et al, "Hypersensitivity to Aloe", ARCH. DERMATOL. 116, at 1064-1065 (Sept. 1980). The untoward effects of yellow sap aloin on human tissue culture is consistent with the inflammatory responses on the intestinal mucosa reported over forty years ago. Melvin W. Green, "The Irritant Effect of Aloin Preliminary Note", AMER. PHAR. ASSOC. 30, at 186-187 (1941). This problem is further compounded by the fact that the yellow sap (aloin) content of individual plants is variable and can vary several hundred percent. E. R. Jansz et al, "The Aloin Content Of Local Aloe Species", J. NATN. SCI. COUN. (Sri Lanka) 9(1) at 107-109 (1981). Jansz et al. specifically report that Aloin [yellow sap] was "commonly used as a laxative".

A second problem associated with processing Aloe vera plants is the effect of darkness on the acid content of the leaves. In the field, leaves are collected and stacked, awaiting processing. The Aloe leaves on the bottom are maintained in darkness. Some leaves may be in darkness for days to weeks before processing. In a matter of hours, the acid content can increase several times. See F. R. Bharucha, "Effect of Prolonged Darkness on Acid Metabolism in the Leaves of Aloe Vera Linn", SCI. AND CULT. 22(7) at 389-390. This affects the taste and effectiveness of the Aloe vera products (too much acid can cause burning or skin irritation).

Coats '372 taught that the aloe plant should be grown under "controlled conditions" to minimize variation in gel substances. However, even under controlled conditions, these substances can vary by more than a hundred percent. Varying mineral content in processed aloe juice, i.e., varying salt content is deleterious to final product preparation in many applications because of lack of consistency of density (e.g. thickness) and even health reasons (burning and irritation because of

substantial salt deviations in final product, e.g., hair shampoo formulations). Chemical stability of aloe products is also affected by varying mineral content of extracted aloe juice. Too much salt in extracted aloe juice can produce separation of phases in final topical aloe formulations. This third problem - consistency in aloe juice composition because of seasonal variations in raw aloe plant mineral content - is brought out by a published report submitted to the National Aloe Science Council on or about November 11, 1982 by The Southwest Institute For Natural Sources, which report is incorporated herein by reference in its entirety, which demonstrates the variable mineral concentration found in Aloe leaves collected from the Rio Grande Valley grown under even controlled conditions. Sodium and chloride ion concentrations vary hundreds of percent, depending on the time of collection. Aloe is a succulent plant and takes up minerals from the soil. During rainy seasons the plants are watered by rain, which is low in minerals. However, during dry seasons, the fields are irrigated with water containing a much higher mineral content. Also, fertilizer adds minerals to the soil and can affect the mineral content. The present invention overcame this problem by dialyzing out the salts in excess concentrations, if they occurred, in order to produce a consistent, final product.

A fourth problem - undesired variations in tonicity of products produced from extracted aloe juice - results from the first three problems just described: (1) The Aloe vera leaves may have a varying yellow sap concentration that can vary in amount and content, (2) acid content, i.e., maleic acid content, within the leaf varies depending on the time the leaf is exposed to light and darkness after cutting, and (3) the mineral content varies with the rainfall and treatment, i.e. fertilizer. These variables affect all the constituents of the Aloe vera gel, such as the Aloe mucilage content. The rate of degradation of the mucilage varies with the acid content, which variation in turn causes the size of the mucilage polymers to vary upon processing. All of these variables affect the osmotic pressure of the

extracted Aloe vera juice. Tonicity determines the degree of moisture transfer of a product to or from skin. Osmotic pressure affects tonicity of Aloe juice (William F. Ganong, REVIEW OF MEDICAL PHYSIOLOGY (Lange Medical Publications, Los Altos, CA) at 16-29, 984 (1983), which can result, in turn, in Aloe products that are isotonic, hypertonic or hypotonic, depending on the nature of the leaves used.

A fifth problem associated with Aloe vera is the degradation of the active substance by hydrolysis. This degradation can take place with time in the presence of water which is the form of the stabilized gel.

It is clear that Henry H. Cobble (U.S. Patent No. 3,892,853) and Billy C. Coats (U.S. Patent No. 4,178,372) in their state-of-the-art processes for processing aloe leaves with the following common characteristics failed to address the aforementioned problems.

1. They added a catalytic amount of a mild oxidant to fresh Aloe vera gel which is brought from about 35°C to about 80°C.

Cobble, col. 2, line 65-68
Coats, col. 2, line 13-16

2. The preferred or oxidant material used for catalytic oxidation is hydrogen peroxide.

Cobble, col. 3, line 16-18
Coats, col. 3, line 43-45

3. Both prefer four to five year old plants.

Cobble, col. 2, line 31-33
Coats, col. 2, line 39-40

4. A non-toxic antioxidant is used to arrest catalytic oxidation, such as tocopherol.

Cobble, col. 10, line 8-9
Coats, col. 5, line 28-31

5. Their mild oxidation is allowed to take place for a period of time to oxidize completely certain gel substances believed to be beta globulin proteins and perhaps alpha globula proteins.

Cobble, col. 3, line 41-44

Coats, col. 3, line 43-47

6. Bill Coats used a commercially available extruder designed for orange juice processing in his process.

Coats, col. 3, line 5-9

7. Neither the Cobble nor Coats process collects and preserves the yellow sap.

8. If the yellow sap contaminates the product, the disclosed process(es) provide(s) no means of removing it.

9. Laboratory analysis of Aloe vera gel prepared by both the Coats and Cobble processes, revealed stabilized Aloe vera gel that varied hundreds of percent in yellow sap content, mineral content, color, taste, gel consistency and osmolarity (tonicity).

In general, both the Cobble and Coats Aloe vera processes do not provide a means for collecting, storing and preserving the yellow sap separately from the mucilage portion. Therefore, when the mucilage is separated from the leaf, it is more prone to be contaminated with yellow sap. Their process(es) require(s) heat and oxidation which can oxidize desirable fractions of the Aloe vera gel as well as undesirable fractions. Additionally, heat accelerates the degradation of the gel. They cannot adjust for the variable mineral content, nor can they select and separate by size, the Aloe vera gel matrix molecule, a substantially acetylated mannan, which affects the moisturizing properties of the gel. Also, the process works best on mature, four to five year old leaves. This is a handicap because a freeze will destroy mature leaves, which has happened in the Rio Grande Valley in three of the last five years. Domestically grown mature leaves are now virtually unattainable in the United States in the present state of the aloe processing industry.

In addition, a problem in the art is that the fraction or chemical substance in Aloe vera that is responsible for its uses has never been definitively identified. For example, in a publication by John Fisher published in the U.S. Pharmacist, August, 1982, at page 37-45, "In contrast to aloe latex, aloe gel

normally does not contain an anthraquinone glycoside except in small amounts. However, a number of substances have been identified in the gel. These substances include mono- and polysaccharides, tannins, steroid compounds, various organic acids and enzymes, saponins, vitamins and minerals such as iron, copper, calcium, magnesium, sodium and potassium. The contribution of these substances to the plant's reputed activity remains unclear". Recently, this has been reported in the Berkeley Medical Newsletter and currently no one has been able to identify any use and associate that with any particular substance in Aloe vera. There is a need to know what the active substance is and to isolate that substance so that one can quantitatively administer a prescribed amount of that substance. For example, aspirin can be obtained from willow bark, but one would not know how much willow bark to chew to get a consistent dosage of aspirin, whereas if the aspirin is extracted out of the willow bark, then one can consistently take the same amount and aspirin in that form is stable.

Fractionation of aloe leaves has not been the source of a great number of publications in the art. Purely academic articles not related to utility of using fractionated portions of aloe have been published by the Indian team of Mandal and Das:

(1) Gaurhari Mandal and Amalendu Das, "Structure of the Glucomannan Isolated from the Leaves of Aloe Barbadensis Miller", (Elsevier Scientific Publishing Company, Amsterdam, 1980) 87 at 249-256.

(2) Gaurhari Mandal and Amalendu Das, "Structure of the D-Galactan Isolated from Aloe barbadensis Miller", (Elsevier Scientific Publishing Company, Amsterdam, 1980) 86 at 247-257.

(3) Gaurhari Mandal, Rina Ghosh and Amalendu Das, "Characterisation of Polysaccharides of Aloe barbadensis Miller: Part III - Structure of an Acidic Oligosaccharide", (Indian Journal of Chemistry, September 1983) 22B at 890-893.

Item (3) is not admitted to be prior art to the present invention.

SUMMARY OF THE INVENTION

The present invention generally relates to the production of refined aloe products, more particularly to the improved processing of aloe plants by separating the various components of the leaves of the aloe plant, and even more particularly to a process for producing an improved mucilaginous substantially anthraquinone-free extract of the aloe plant which is pH stable, substantially consistent in salt content, and capable of achieving a predetermined level of tonicity.

The present invention is also directed to a second process whereby the active chemical substance in the aloe plant is physically extracted from whole leaves or the improved mucilaginous substantially anthraquinone-free extract. According to this process, the chemical substance is further freeze dried or lyophilized and optionally sterilized by irradiating it with either gamma or microwave radiation. In this form the chemical substance is substantially non-degradable and can be given in a prescribed amount.

The present invention is also directed to the active chemical substance in the aloe plant in the form produced by the second process described above. The active chemical substance has been found to be a substantially non-degradable lyophilized ordered linear polymer of acetylated mannose monomers. The mannose monomers are preferably bonded together by β $(1 \rightarrow 4)$ bonds. The active chemical substance has been measured, standardized and characterized by analytical chemistry and bioassay techniques.

By "substantially anthraquinone-free" as that term is applied to gel, fillet(s), extract, or the like, I mean less than about 0.05 percent by weight. The mucilaginous substantially anthraquinone-free extract of my invention preferably has less than about 0.001 percent by weight of anthraquinone. By "pH stable" I mean for an extract from a predetermined Aloe fraction grown in a predetermined locale and Aloe species having a reproducible pH within one-half pH unit over a three year period. By the term "substantially free in salt content" I mean that property for an extract (from a like predetermined aloe fraction

grown in a predetermined locale and aloe species) a reproducible salt content within ± 20 ppm over a three year period. By "predetermined tonicity" I mean that tonicity for an extract from a predetermined aloe fraction having a reproducible osmotic pressure of ± 20 mOsm over a three year period. By "active chemical substance" I mean that substance which is responsible for the wound healing and other beneficial properties of Aloe vera. By "substantially non-degradable" I mean a product which decreases in molecular weight by less than 5 percent over a period of two years and a product which maintains more than 95 percent of its biological activity over a period of two years. By "substantially acetylated mannan" I mean partially or substantially completely acetylated mannose monomers.

For example, for a topical product it is desired to have a pH stable product that matches the natural acid mantle of the skin (pH from 4 to 6). For broken skin the pH should more closely resemble serum or plasma (pH about 7.4). This level is adjusted for each skin care product based upon intended use. Total salt content should be such to produce an osmotic pressure between about 100 or about 500 milliosmoles (mOsm). Oily skin, for example, needs isotonic to hypertonic solutions (280-500 mOsm); normal skin from 180-380 mOsm; dry skin from 100-280 mOsm.

More particularly, the process of my invention is one for "that property extracting substantially anthraquinone-free" aloe gel from a leaf of the aloe plant, which process comprises the following steps:

(a) washing an aloe leaf in a bactericidal solution to remove substantially all surface dirt and bacteria;

(b) removing at least a first end portion from said washed leaf;

(c) draining and collecting anthraquinone rich sap from said cut and washed leaf; and

(d) removing rind from said leaf to produce a substantially anthraquinone free gel.

Removal of "substantially all surface dirt and bacteria"

means (1) removal of dirt to the extent that remaining dirt is less than 0.1% by weight of the weight of the leaf and (2) killing such surface bacteria that the remaining surface bacteria are less than 100 count per gram of leaves.

One skilled in the art will appreciate that instead of steps (b), (c) and (d), one may instead (b) crush the washed aloe leaves and (c) dialyze the crushed leaves chemically removing unwanted fractions, i.e., anthraquinones, minerals and acids and the rind to produce a substantially anthraquinone free gel. More preferably, aloe leaves or whole plants may be collected from the field sufficiently clean to eliminate a washing step; the juice is extracted by crushing, squeezing and/or extrusion, and the desired aloe fraction components, e.g. free of anthraquinones, extracted from the whole juice using dialysis or ultrafiltration as described herein. The juice fractions can be separated into individual components and subsequently remixed in any desired combination, and optionally stabilized as described herein.

Preferably, my process for producing substantially anthraquinone-free juice from the leaf of an aloe plant comprises the following steps:

(a) washing an aloe leaf in a bactericidal solution to remove substantially all surface dirt and bacteria;

(b) removing a first end portion and a second portion from said washed leaf;

(c) draining and collecting anthraquinone rich sap from said washed and cut leaf;

(d) removing rind from said leaf to produce a substantially anthraquinone-free aloe gel fillet; and

(e) grinding and homogenizing said substantially anthraquinone-free aloe gel fillet to produce substantially anthraquinone-free juice.

Furthermore, my preferred process may further comprise the step of ultrafiltration in order to osmotically adjust the aloe juice or aloe vera fraction, or to reduce even further the levels of anthraquinones to less than 5 ppm, even down to less than 5 ppm, even down to less than 100 parts per billion by

weight.

These steps enable the processor to use large or small leaves even less than one year old because the polymer size found in the mature leaves can be selected and processed out of smaller, immature leaves.

One of the advantages of the instant process is that damaged leaves previously considered unusable due to strong winds or poor collection techniques can be processed, and the undesirable contaminants can be dialyzed out.

Most preferably, my process envisions further filtering the aloe juice to remove fibrous material. In such form, the aloe juice may be incorporated in an abundant number of different products for internal or external use.

The process of my invention may further comprise, as a preferred embodiment, the addition of one or more members selected from the group consisting of preservatives, flavorants, or any FDA approved additives (generally recognized as safe, or "GRAS" additives) to said aloe gel fillet or said aloe juice or said Aloe vera fraction. Most preferably, the preservative is selected from the group consisting of benzoic acid, potassium sorbate, methyl paraben and propyl paraben or any other FDA approved additive for topical or internal uses.

The juice of my invention, optionally, may be irradiated, freeze dried, sonic sterilized or pasteurized, whereby said juice is preserved.

The ultrafiltration (dialysis) step incorporates membrane technology that allows the selection of filters with different pore sizes, depending on the condition of the cut aloe leaves, that can accomplish any combination of the following:

(1) A small pore size filter (preferably 100 Daltons) that separates out water and salts from the Aloe vera gel, if needed.

(2) Larger pore size filters (preferably 500 Daltons) that can separate out the acids from the Aloe vera gel, if needed.

(3) Even larger pore size filters (preferably 2000

Daltons) that can separate the yellow sap components from the Aloe vera gel, if needed.

(4) And even larger pore size filters (preferably from 10,000-100,000 Daltons that can size the gel matrix polymers, and divide them out by molecular weight.

A Romicon® 4-column (Romicon Co., 100 Cummings Park, Woburn, MA 01801, Model No. HF4 SSS, Membrane Type PM50, Membrane Nos. H526.5 - 43 - pm50) as an ultrafiltration device is recommended.

As an additional preferred embodiment, the washing step of the process may comprise washing the substantially anthraquinone-free aloe gel fillet in a tumbler washer prior to grinding said fillet.

An effective dermal wound gel may be produced by the process of my invention, and may perferably comprise 0.1 to 100 weight percent substantially anthraquinone-free aloe juice, 0 to 2 weight percent allantoin, 0 to 6 weight percent panthenol, and excipients or carriers. See Example 7. Levels of allantoin and/or panthenol are controlled by the FDA. See Zimmerman, THE ESSENTIAL GUIDE TO NONPRESCRIPTION DRUGS (Harper and Row, New York, 1983).

A second process of my invention is one for extracting the active chemical substance in the aloe plant from a leaf of the aloe plant, which process comprises the following steps:

(a) washing an aloe leaf in a bactericidal solution to remove substantially all surface dirt and bacteria;

(b) removing at least a first end portion from said washed leaf;

(c) draining, preserving and collecting anthraquinone rich sap from said cut and washed leaf;

(d) removing rind from said leaf to produce a substantially anthraquinone-free aloe gel fillet;

(e) grinding and homogenizing said substantially anthraquinone-free aloe gel fillet to produce substantially anthraquinone-free aloe juice having solubilized matter;

(f) adding a water soluble, lower aliphatic polar

solvent to the aloe juice to precipitate the active chemical substance and thereby to form a heterogeneous solution;

(g) removing the water soluble, lower aliphatic polar solvent and the solubilized matter from the heterogeneous solution to isolate the precipitated active chemical substance; and

(h) drying the precipitated active chemical substance.

One skilled in the art will appreciate that instead of steps (b), (c) and (d), one may instead (b) crush the washed aloe leaves and (c) dialyze the crushed leaves chemically removing unwanted fractions, i.e., anthraquinones, minerals and acids and the rind to produce a substantially anthraquinone-free gel that may then be subjected to steps (e), (f), (g) and (h) to extract the active chemical substance.

One skilled in the art will appreciate that one may simply obtain aloe juice having solubilized matter from aloe leaves and then subject this juice to steps (f), (g), and (h) to extract the active chemical substance.

Indeed, one skilled in the art will also appreciate that instead of steps (b), (c), (d) and (e), one may instead crush the washed aloe leaves and extrude anthraquinone-rich aloe juice having solubilized matter and then subject the aloe juice to steps (f), (g) and (h) to extract the active chemical substance. The active chemical substance is effectively separated from anthraquinones and deleterious ions by this process since all the anthraquinones and all the ions are water soluble and remain in the liquid solvent phase and do not precipitate.

One skilled in the art will also appreciate that instead of steps (b), (c), (d) and (e) one may instead grind the whole washed aloe leaves, filter out fibrous material, and homogenize the remainder to produce anthraquinone-rich aloe juice having solubilized matter. The aloe juice can then be subjected to steps (f), (g) and (h) to extract the active chemical substance. The active chemical substance is effectively separated from anthraquinones and deleterious ions by this process for the reasons noted above.

One skilled in the art will appreciate that an addi-

tional process for extracting the active chemical substance in the aloe plant from a leaf of the aloe plant comprises the following steps:

a) washing an aloe leaf in a bactericidal solution to remove substantially all surface dirt and bacteria;

b) removing rind from said leaf to produce an aloe gel fillet;

c) grinding and homogenizing said aloe gel fillet to produce aloe juice having solubilized matter;

d) adding a water soluble, lower aliphatic polar solvent to the aloe juice to precipitate the active chemical substance and thereby to form a heterogeneous solution;

e) removing the water soluble, lower aliphatic solvent and the solubilized matter from the heterogeneous solution to isolate the precipitated active chemical substance; and

f) drying the precipitated active chemical substance.

As noted above, the active chemical substance is effectively separated from anthraquinones and deleterious ions by this process since all the anthraquinones and all the ions are water soluble and remain in the liquid solvent phase and do not precipitate.

Removal of "substantially all surface dirt and bacteria" means (1) removal of dirt to the extent that remaining dirt is less than 0.1% by weight of the weight of the leaf and (2) killing such surface bacteria that the remaining surface bacteria are less than 100 count per gram of leaves.

Preferably in all the above processes for extracting the active chemical substance in the aloe plant from a leaf of the aloe plant, four volumes of the water soluble, lower aliphatic polar solvent are added to one volume of aloe juice to precipitate the active chemical substance. Preferred water soluble, lower aliphatic polar solvents are methanol, ethanol and propanol. The most preferred solvent is ethanol. One skilled in the art will recognize that other water soluble, lower aliphatic polar solvents may be substituted for the preferred solvents as long as the active chemical substance will precipitate

therefrom.

It is preferred in the above extraction processes that the active chemical substance is allowed to precipitate from the water soluble, lower aliphatic polar solvent and aloe juice mixture for about four hours. It has been determined that allowing the mixture to precipitate for four hours gives the optimum yield of active chemical substance and that after four hours the precipitated active chemical substance begins to degrade. It has also been determined, however, that significant amounts of active chemical substance have been recovered after a precipitation period of 24 hours. One skilled in the art will appreciate that the optimum precipitation time period is dependent on ambient temperature and pressure as well as the nature of the water soluble, lower aliphatic polar solvent.

It is also preferred in the above extraction processes that the precipitated active chemical substance be dried by lyophilization rather than by oven drying since heat may aid in the hydrolysis or degradation of the active chemical substance.

In all of the above extraction processes, any fibrous material (cellulose) contained in the aloe juice is also precipitated by the water soluble, lower aliphatic polar solvent but is precipitated early with the addition of the solvent and is less dense than the active chemical substance. The fibrous material remains on the surface of the solvent after the active chemical substance has been allowed to settle and can therefore be removed quite easily. One skilled in the art will appreciate that one may instead filter the aloe juice to remove fibrous material prior to the addition of solvent.

More preferably, in all of the above processes, aloe leaves or whole plants may be collected from the field sufficiently clean to eliminate a washing step.

The dried, precipitated active ingredient, optionally, may be irradiated by gamma or microwave radiation whereby said active chemical substance is sterilized and preserved.

Accordingly, the present invention provides new and improved methods for the production of aloe vera products.

The present invention furthermore provides improved methods for processing the leaf of the aloe vera plant in a manner which avoids the undesirable combination or mixture of distinctively characteristic portions of such plant leaf.

The present invention moreover provides improved processes for preparing various extracts of the leaf of the aloe vera plant which minimizes the concentrations of undesirable components in the finished extracts.

The present invention also provides new and improved processes for preparing extracts of the leaf of the aloe vera plant which maximize the concentration of certain components characteristic of particular portions or segments of the leaf while minimizing or eliminating certain components characteristic of other portions or segments of the leaf.

The present invention also provides new and improved processes for preparing extracts from the leaf of the aloe vera plant which are low in concentration of yellow sap of the leaf.

My preferred processes have these advantages over the prior art:

1. No catalytic amount of a mild oxidant is used, thus preventing the oxidation of desirable constituents.

2. The processes do not require heat, but can be performed at room temperature.

3. Young, immature or damaged leaves can be used.

4. An antioxidant does not have to be used to arrest catalytic oxidation.

5. The beta gobulins and alpha globulins can be dialyzed out.

6. Both hand and mechanical extruders can be used.

7. The processes allow for the separate collection, storage and preservation of the yellow sap. (The yellow sap has been banned as a laxative, but it can be used as a sunscreen on undamaged skin. Plus, it provides a tan color to the skin.)

8. If the yellow sap gets into the Aloe vera gel, it can be removed.

9. It can provide Aloe vera gel that is standardized

in its yellow sap content, mineral content, color, taste, gel consistency and osmolarity (tonicity).

10. Individual components of Aloe vera gel can be isolated, dialyzed out and concentrated to concentrations many times greater than is found in the natural juice.

11. Also, by separating out the fractions, new combinations of Aloe vera gel components are now attainable that heretofore did not exist in nature.

12. In the event that too much preservative, flavoring or other GRAS substance is overadded, the batch can be saved in most cases by dialyzing out the excess.

13. Selected Aloe components can be dialyzed out, chemically altered and then added back.

14. Enzymes can be added to the Aloe gel, allowed to react and then separated out by ultrafiltration.

The present invention finally provides a method for extracting the active chemical substance in Aloe vera gel. This active chemical substance shall hereinafter be referred to as Carrisyn®. As mentioned above, Carrisyn® has been found to be a substantially non-degradable lyophilized ordered linear polymer of acetylated mannose monomers which is measured and standardized both using analytical chemistry and bioassay techniques.

DESCRIPTION OF THE FIGURES

Figures 1 and 2 show cut-away portions of an aloe vera leaf.

Figure 3 shows a schematic of a preferred leaf washing apparatus used in my process.

Figure 4 shows a schematic of a preferred apparatus for sectioning and soaking of aloe vera leaves.

Figure 5 shows a schematic of preferred apparatus for the cutting of aloe sections into fillets and for coarse grinding.

Figure 6 shows a schematic of preferred apparatus for fine homogenization and (optional) filtering.

Figure 7 shows a schematic of a preferred use of dialy-

sis equipment for fine separations of processed aloe material.

Figures 8-13 show infrared spectra for different samples of Carrisyn®.

Figure 14 shows an infrared spectrum for raw aloe gel that was cast into a film.

Figure 15 shows an infrared spectrum for a sample of Carrisyn®.

Figures 16-17 show infrared spectra for a sample of Carrisyn® with a one hour and a ten hour time period between the time the aloe gel was removed from the leaf and the time the aloe juice was extracted with alcohol.

Figure 18 shows a plot of Carrisyn® yield versus reciprocal time of gel in alcohol (precipitation process).

Figure 19 shows a plot of Carrisyn® yield versus reciprocal total process time before precipitation.

Figure 20 shows a plot of Carrisyn® yield versus reciprocal time of homogenization and filtration.

Figure 21 shows an infrared spectrum of the alcohol precipitation product of the juice from Aloe ferox.

Figure 22 shows an infrared spectrum of the alcohol precipitation product of the juice from Aloe africana.

Figure 23 shows an infrared spectrum of the alcohol precipitation product of the juice from Africana ferox.

Figure 24 shows an infrared spectrum of the alcohol precipitation product of the juice from Aloe dichotoma.

Figure 25 shows an infrared spectrum of cellulose.

Figure 26 shows an infrared spectrum of polygalacturonic acid.

Figure 27 shows an infrared spectrum of glucomannan from the Konjac plant.

Figure 28 shows an infrared spectrum of dextran.

Figure 29 shows an infrared spectrum of guar gum.

Figure 30 shows time course of ^{51}Cr release by topical agents. Cultures of human fibroblasts were incubated for various times with 0.05% Granulex®, Hibiclens®, Betadine® or Carrisyn® (CDWG) and the percent of the total radioactivity released was

determined. Data are means of 3-5 separate determinations at each time point. Control cells (treated with media alone) released 3-5% of the total ^{51}Cr during 30 minutes.

Figure 31 shows influence of concentration on cell injury. Cultured fibroblasts were incubated with varying concentrations of Hibiclens®, Granulex®, Betadine® or Carrisyn® (CDWG) for 15 minutes at 37°C. The percentage of ^{51}Cr released was determined for each concentration. Control release (from untreated cells) ranged from 1-5%. Data are means of 3-5 separate determinations.

Figure 32 shows release of chromium: effect of CDWG and serum. Cells were incubated in medium alone (dark bars) or in medium containing Carrisyn® (0.5%) (hatched bars) or medium with 10% fetal calf serum (strippled bars) for 15 minutes. Betadine®, Granulex® or Hibiclens® (0.15%) were added and the incubation was continued for 15 minutes longer. Data are means of 3-4 separate experiments.

DETAILED DESCRIPTION OF ADDITIONAL PREFERRED EMBODIMENTS

In accordance with these and other objects, the process of the present invention arises out of the discovery and recognition that particular distinct portions of the aloe vera leaf, specifically the yellow sap, internal gel matrix, and outer rind, exhibit characteristics respectively unique to those portions; and that the characteristics of one distinct portion may be conducive to its use for a particular application or end use product with the characteristics of another distinct portion being inconsistent with, or detrimental to, such use. For example, it has been determined that an extract derived from the internal gel matrix has characteristics which make it extremely desirable in the production of cosmetic or skin care products; but the yellow sap has characteristics which are particularly detrimental for such use.

Furthermore, I have discovered and recognized that sub-portions of the yellow sap and internal gel matrix have characteristics which are even unique to those sub-portions, the extracts therefrom therefore having potentially distinct uses from one another. A summary of these distinct portions, and some of their characteristics and potential uses as I have found, is as follows:

PORTION	SUB-PORTION	UTILITIES
Yellow Sap	(1) Sediment	laxative, antifungal, antibiological, pesticidal and sunscreen
	(2) Supernatant	mucosal protective action, sunscreen
Internal Gel Matrix	(1) Mucilage	penetrant, hypoallergenic, moisturizer
	(2) Gel Fillets	ulceroprotective, cell stimulative
		moisturizer, wound healing
	(3) Interstitial Fibers	natural preservative, hemostat
	(4) Residual Matrix	cell growth stimulant
Outer Rind		pesticidal insect repellent, paper pulp fiber

Based upon the aforementioned knowledge and recognition, and in order to optimize the quality and concentration of the desired components in the final extract depending upon the intended use thereof, the process of this invention is directed

to the initial fractionating of the leaves of the aloe vera plant into the particular distinct portions and sub-portions defined above as well as the separation and isolation of particular components of such sub-portions defined above. The specific details and features of such processes will become more readily understood and appreciated from the following detailed description. The present invention is also directed to particular components that are isolated by the above-mentioned processes.

FRACTIONATION PROCESS

The extracts produced by the process of the present invention are preferably obtained from the outer, lower leaves of mature, outdoor grown aloe vera plants. A two year old plant is normally mature; however, the broader leaves of a four or five year old plant typically contain larger amounts of the desired extracts and are also easier to handle. Four or five year old leaves of Aloe barbadensis Miller grown in the Rio Grande Valley of Texas are most preferable. These leaves generally weigh about 1½ - 2½ pounds each. Depending upon the particular use or products that are desired, the leaves can be processed immediately after cutting them from the plant or they can be stored under appropriate conditions for varying time periods before they are processed. Additionally, concentration of various components of the leaf are affected by seasonal variations and the environmental conditions to which the leaves are subjected, all of which should be taken into consideration depending upon the specific intended use to which the plant extracts are to be directed.

The leaves should be pulled or cut from near the base of the plant, preferably without breaking or damaging any part of the leaf prior to processing. One preferably employs a small knife of less than 6 inches, e.g., a pocket knife and cuts the leaf at the base immediately above the stalk, and peels the leaf from the stalk, in order to prevent leakage of the clear cellular gel or contamination of the gel with the yellow sap. Any breakage or bruising of the leaf can result in the undesirable commingling of the distinct portions, and therefore component characteristics, of the leaf.

After removal from the plant, the leaves are normally cleaned by washing them with a mild scrubbing action or spraying with a suitable detergent solution (e.g., we prefer OLYMPIC POOL CHLOR 65®, distributed by York Chem Co., Dallas, Texas). In some instances, the cleaning takes place with the aid of soft brushes. After cleaning, the leaves are rinsed thoroughly in clean water to remove any vestige of any detergent solution.

The bottom white or light colored portion of each leaf and the tipmost portion thereof are removed by cutting carefully with a small sharp knife. These portions, essentially constituting the ends of the leaf, may be separately processed to obtain the yellow sap therefrom for those applications in which it is desired to produce products having components with the yellow sap characteristics referred to above.

The remaining portion of each aloe vera leaf is then crosscut into short segments, preferably one-half inch in length, and each segment is placed upright in an aqueous solution (preferably deionized water) which may be hypertonic, isotonic or hypotonic, resulting in the yellow sap draining from the segments. Alternatively, in those applications in which it is desired to collect the yellow sap for use in other preparations having the characteristics noted above, the segments may be placed upright in a dry collection container, preferably of stainless steel with a stainless steel wire mesh bottom to allow drainage and for water contact to allow the water to dialyze the leaves.

The segments are permitted to drain for approximately twenty to thirty minutes in this manner. The cut segments will eventually form a seal and cease draining. The yellow sap that is collected will then, upon standing for an appropriate period of time, separate into two sub-portions, namely sediment and supernatant, respectively. The yellow sap is useful for making a good sunscreen on intact skin (<u>not</u> broken skin) and provides the skin with an olive tan color, and is also useful for the manufacture of laxatives.

After completion of the procedures which remove the

yellow sap from the cut leaf segments, the segments are then pared to form fillets utilizing any suitable equipment such as a wire (i.e., consumer cheese) slicer or paring blades (e.g., paring knife) to remove the outer rind or skin of the leaf segments and the layer that is immediately below such outer skin. The leaf segments may be frozen to facilitate this skinning procedure. After paring, what remains is the internal gel matrix portion (fillet), and this portion is inspected and hand cleaned to remove any adhering skin or discolored portions to eliminate any residual yellow sap therefrom. One uses a mild water spray, preferably deionized water (and free of alcohol) or submerges the gel matrix portion under flowing clean water, to facilitate removal of such yellow sap residuum.

The resultant fillet (internal gel matrix) can then be drained for approximately an hour. During this draining procedure, a slimy coating usually forms on the surfaces of the gel matrix, this coating being collected by gravity or assisted by appropriate means such as centrifugation. This collected coating is the mucilage sub-portion referred to above.

The remainder of the gel matrix, in the form of gel matrix strips, may then be ground, shredded or blended to break up the interstitial fibers present therein, or the gel matrix strips may be forced through a wire mesh or filter screen in order to achieve liquefaction. This resultant substance may then be subsequently homogenized. Alternatively, the gel matrix strips may be frozen and thawed and subsequently mixed to produce a liquid substance with fibers (such substance constituting the gel fillets sub-portion referred to above). This substance can then be filtered to obtain the interstitial fibers sub-portion, leaving the residual matrix sub-portion referred to above. In short, for topical applications the interstitial fibers are removed from the internal gel matrix portions (fillets), while for internal applications the fibers are maintained with the balance of the fillet contents. For topical or internal use the appropriate gel fractions are homogenized, e.g., by a dairy milk homogenizer (prior to addition of stabilizers, preservatives,

excipients, color or other GRAS additives) in order to make the gel fractions more accessible to further processing, e.g., to reduce growth of bacteria. At this point in the process, the resulting extract may be tested utilizing known quality control procedures such as mass spectroscopy, gas chromatography, high pressure liquid chromatography, or other analytical techniques.

The homogenized extract thus obtained typically has a pH of approximately about 4 to about 5, preferably about 4. To increase its stability, the homogenized extract is typically normalized by the addition of citric acid, boric acid, ascorbic acid, or other suitable materials depending on end use (e.g., for internal consumption, citric acid and/or ascorbic acid is employed; for eye applications boric acid is used). Finally, the homogenized extract may be sterilized, utilizing any method well known in the art such as irradiation, sonic sterilization, controlled heating or by the addition of antibacterial or bacteriostatic chemicals such as GRAS - cetyl alcohol, sodium benzoate or ethyl alcohol, in amounts and concentrations well known in the art. It will be appreciated that various combinations of the foregoing may also be utilized to sterilize the extract produced in accordance with this invention. It will also be appreciated that various industry acceptable stabilizers and additives may be added to the homogenized gel extracts if desired.

Final quality control procedures may be utilized at this point and the extract is then ready for bottling or compounding with other materials. The extract from the internal gel matrix, when prepared as described herein, will contain a very low concentration of yellow sap and is most suitable for skin care and cosmetic applications.

All steps in the process described are performed at about room temperature.

Various modifications of the disclosed process and compositions of the invention, as well as alternative modifications, variations and equivalents will become apparent to persons skilled in the art upon reading the above general description. The following examples are illustrative only and are not intended

to limit the scope of the appended claims, which cover any such modifications, equivalents or variations.

EXAMPLE 1

Process for preparing stabilized aloe gel fillets, and homogenization.

A. PRELIMINARY WORK:

1. Previously cleaned tanks, mixers and fittings were sanitized with 50% isopropyl alcohol (IPA) solution and rinsed free of IPA with hot D.I. Water.

2. Pumps and attached hoses were drained with 5% "HTH" chlorine swimming pool solutions, then flushed with water.

3. The pumps and attached hoses were sanitized with 50% isopropyl alcohol solution. Pumps and attached hoses were flushed with hot deionized water until free of IPA.

4. An homogenizer and attached hoses and pumps were sanitized with 50% isopropyl alcohol solution. The homogenizer and attached hoses were flushed with hot deionized water until free of IPA.

Aloe barbadensis Miller leaves collected from the Rio Grande Valley were transferred in a refrigerated truck at 40 to 45°F within eight hours after harvest and stored under refrigeration at 40 to 45°F until processed to reduce degradation.

Twenty to sixty pounds of stored leaves were then placed in a prewash bath of an aqueous solution of calcium hypochlorite at room temperature substantially to remove surface dirt from the leaves and kill surface bacteria on the leaves. The aqueous solution of calcium hypochlorite was prepared by adding approximately 0.125 grams of 98% calcium hypochlorite to one liter of water to produce a solution containing 50 ppm of free chlorine. The leaves remained in the prewash bath for a period of approximately five minutes.

Next, the substantially dirt and bacteria free leaves were placed on the horizontal conveyor belt of a "Thompson Aloe Washer" made by Thompson Manufacturing Company, Harlingen, Texas. The "Thompson Aloe Washer" washed the leaves with room temperature water to remove the surface dirt and aqueous solution of

calcium hypochlorite from the leaves. Again, the leaves were visually inspected and hand scrubbed as necessary to remove any surface dirt remaining on the leaves. Such leaves were then rinsed with room temperature water.

The tip and butt portion was then removed from each leaf and the leaves were placed in stainless steel basket-type containers, placed together on top of a funnel shaped stainless steel collector, each container having a mesh bottom. Yellow sap was allowed to drain from the leaves for approximately 30 minutes. The yellow sap passed through the mesh bottom of the stainless steel basket and was collected in a funnel shaped collector.

The stainless steel basket type containers containing the aloe leaves were removed from the collector, and were then submerged in a second stainless steel vessel comprising a room temperature water bath of continuously horizontally flowing rinse water moving counter-current to the containers which are slowly moved by hand from one end of the vessel to the other, for approximately thirty minutes to one hour. This allows the yellow sap to drain further from the leaves. The leaves must be allowed to soak in this solution for 30 minutes.

The leaves were then removed from this solution and the rind was removed from each leaf with a sharp knife or wire cheese slicer to produce an aloe gel fillet from each leaf portion. The aloe gel fillets were visually inspected and any contaminated aloe gel fillets or fillet part detected by a characteristic yellowish discoloration were discarded. The total mass of uncontaminated aloe gel fillets was 20 to 60 percent of the starting leaf mass depending on the leaf size and condition.

The uncontaminated aloe gel fillets were then placed in a 750 seat restaurant set stainless steel garbage disposal unit which coarse ground the fillets to an average particle size of the consistency of a thick, but freely flowing (coarse homogenized) liquid. The stainless steel garbage unit was made by the N-sink-erator Division of Emerson Electric Co., Racine, WI, Model No. SS-150-13, Serial No. 115132.

The coarse ground aloe gel fillets then passed to a stainless steel holding vat. The holding vat was made by Process Equipment Corp. of Belding, Michigan, Model No. 100 gallon OVC, Serial No. 40865-3. In this holding vat the coarse ground aloe gel fillets were mixed with a GRAS preservative such as sodium benzoate, potassium sorbate, methyl paraben or propyl paraben, depending on use and ratio of final product oil to aloe fraction. For topical use a mixture of methyl and propyl paraben is recommended; for internal use a mixture of sodium benzoate and potassium benzoate is recommended. For example, if one has a topical application using 10% oil, 90% Aloe fraction, the ratio of propyl paraben to methyl paraben is 1:9. The weight concentration ratio of the coarse ground aloe gel fillets to the preservative was approximately 1000:1, approximately the upper limits of the FDA guidelines for food and cosmetic preservatives. The preservative solution in the holding vat was initially prepared by adding approved quantities of the GRAS preservative to 10 gallons of deionized water.

From the holding vat, the coarse ground aloe gel fillet - preservative solution was pumped to a homogenizer. The homogenizer was made by Crepaco Food Equipment and Refrigeration, Inc., of Chicago, Illinois, Serial No. 04-03. The homogenizer was of a type typically used in dairy processes for the homogenization of milk. The coarse ground aloe gel fillet - preservative solution was finely homogenized under a pressure of 200 to 10,000 psi (1.38 to 68.9 MPa).

From the homogenizer the finely homogenized aloe gel fillet - preservative solution was pumped to a stainless steel storage tank. The storage tank was made by Process Equipment Corp. of Belding, Michigan, Model No. 1000 gallon OVC, Serial No. 40866-2. The total mass of the homogenized aloe gel fillet - preservative solution was 20 to 60 percent of the starting leaf mass. Then, if necessary, the homogenized product was dialyzed using ultrafiltration.

Figures 3-7 disclose in even further detail preferred embodiments of the instant process. Specifically, in Figure 3,

there is disclosed apparatus for leaf washing. Aloe vera leaf washing equipment (Thompson Manufacturing Company, Harlington, Texas) A is utilized whereby leaves are first presoaked in vat 4. The whole leaves α are then placed by hand onto a conveyor belt 8 by which they are pulled underneath two brushes 9a and 9b. Conveyor belt 8 is driven via a chain 7 which is rotated on a second end by motor and pulley 6 which extends from a housing 5, also provided by Thompson Manufacturing Company. As the leaves are brushed and washed, upon passing through the second brush 9b the leaves are inspected at the end 10 of conveyor belt 8, by which the leaves are visually inspected and determined whether or not they are sufficiently clean. If the leaves are not sufficiently clean, they are placed into vat 12 for further washing; if the leaves are sufficiently clean, they are placed upon an upwardly moving conveyor B having steps 13 for which each individual leaf may be further washed with tap water by sprayers 11. The conveyor B is provided by Dallas Mill Wright Co. of Seagoville, Texas. The rinse sprayers 11 are provided by Key Plumbing, Seagoville, Texas. Stainless steel vat 12 is made of 316 stainless steel and is custom made by the National Sheet Metal Company of Dallas, Texas.

After washing, as indicated in Figure 4, the leaves are sectioned and soaked. After moving up through steps 13 of conveyor B, the clean, raw leaves α drop onto a tray 14 which is provided with a hole 15 for removal of trash. Tray 14 is part of a sectioning and soaking apparatus C of 316 stainless steel provided by the National Sheet Metal Company of Dallas, Texas. This equipment is custom fabricated. On tray 14 the leaves are manually sectioned at both ends with the tips and butts disposed through the hole 15 into a trash receptacle (not shown). The cut leaves β are then stacked into any one of a number of baskets 16 of stainless steel each having a wire mesh bottom made of stainless steel. Then these baskets 16 are placed in a stainless steel track which forms the top part of a trapezoidal funnel 17 by which yellow sap is drained from the bottom of the leaves through the baskets, falling into the bottom portion of the fun-

nel 17. The yellow sap is periodically removed and is kept frozen for storage. The yellow sap draining step takes about 30 minutes.

After this step, the cut leaves β, still retained in basket 16 are manually moved to water bath 18 at positions closest to trapezoidal funnel 17. In countercurrent flow, water comes into bath 18 through inlet water pipe 19, at a point farthest removed from trapezoidal funnel 17 and is subsequently removed through exit water pipe 20 at a position closest to trapezoidal funnel 17. Trays are gradually moved manually through the water bath in a direction away from trapezoidal funnel 17, and the washing step whereby the baskets remain in water bath 18 takes approximately one hour.

After washing, the baskets are placed on tray 21 for drying, which lasts for only a few minutes. Again, the entire assembly in Figure 4, pertaining to the baskets, including wire mesh, yellow sap draining and auto washing equipment is made of 316 stainless steel custom fabricated by the National Sheet Metal Company, Dallas, Texas.

After washing, the cut leaves β stacked in basket 16 on tray 21 are then moved to an area for further sectioning into fillets, as shown in Figure 5. Rind 22 is removed from the fillets so that only substantially clear material remains. The rest of the rind 22 is discarded. Fillets γ are then placed into trough 23 which feeds to a rough coarse grinder D. The trough 23 is manufactured of 316 stainless steel by the National Sheet Metal Company of Dallas, Texas. The grinder is a Model No. 5150-N-Sink-erator (Watson Food Service Industries, Inc., Dallas, Texas). After rough coarse grinding through grinder D, the processed material γ ' emerging from the grinder passes through to a portable tank E of approximately 100 gallon capacity which comprises a vertical single shell tank of 316 stainless steel (Perma-San, Manufacture, distributed through Van Tone, Inc., Dallas, Texas). Coarse ground fillet γ ' is agitated in tank E by a Perma-San agitator (Model No. AAPH2) also distributed through Van Tone, Inc. of Dallas, Texas.

After rough homogenization, material from tank E is taken to a separate staged area for fine homogenization and (optional) filtering. In Figure 6, material from tank E is pumped through pump 26 (centrifuge pump provided by Crepaco, Inc., Dallas, Texas, Model No. 4V-81, of stainless steel) driven by Reliance Motor Model No. B76Q2139M-VF of the Reliance Electric Company, Cleveland, Ohio into a fine homogenizer F (Crepaco, Inc., Dallas, Texas, Model No. 3DD13). After fine homogenization the material is transported to a large 1,000 gallon vertical single shell mixing tank G made of 316 stainless steel (Perma-San, Manufacturer, distributed to Van Tone, Inc. of Dallas, Texas). The finely ground fillet Δ is agitated by a Perma-San agitator 26a (Perma-San, Model No. AAPH2, distributed through Van Tone, Inc., Dallas, Texas). Material Δ in tank G may be sent directly to processing for a drinking product for direct human consumption after flavoring and appropriate preservatives are added. In the alternative, material in tank G may be removed and sent to pump 27a which forms one unit with filter 27b for removal of pulp through discard line 28. Pump 27a and filter 27b form the part of a Lomart diatomite filter (Model No. 99-2138 distributed by Allen Recreation Company, Dallas, Texas). Filtered material is then pumped into tank H, which like tank E, can be provided with a lid 25.

In Figure 7, finely ground fillet Δ, is partially filtered, is stirred by mixer 29 and pumped through pump 30 into a dialyzer. Mixer 29 is a Perma-San agitator (Model No. AAPH2) distributed through Van Tone, Inc. Company, Dallas, Texas. Pump 30 is a Superior stainless steel Model SCS45 process pump (Superior Stainless Company, Delavan, Wisconsin). Attached to process pump 30 is motor 30a of 3 horsepower made by Baldor Motor of 4450 rpm (Cat. No. CM3559T of the Baldor Electric Company, Ft. Smith Arkansas). Material pumped through pump 30 passes through dialysis unit J, (a Romicon Model HF4SSS ultrafiltation system made by Romicon Inc., Woburn, Massachusetts) having 4 filters 31 (not shown) each filter housed in filter housing 32. Material passes vertically to a point where portions can be removed

through separation lines 34 and into separation discharge lines 35. Other material not separated is recycled through recycle return line 33 back into the dialysis unit, or in the alternative, through separation return line 36 back into the vat I.

Depending on the fraction of aloe desired and end product sought, the desired material can either be obtained through separation discharge line 35 after processing or in vat I. For example, if excess water and minerals need to be removed, a small pore size ultrafilter can be used to separate out the water and minerals which are discharged through line 35 and the desired aloe fraction is returned to vat I. This process can be repeated until the desired amount of salt and water is removed from the product contained in vat I simply by circulating it through the dialysis unit. This process can include more than one dialysis step. For example, as previously described, the salts, low molecular weight acids and unwanted anthraquinones can be removed in a first dialysis step. The unwanted material is discharged through separation discharge line 35 and the desired fraction is returned to vat I. This step is accomplished by using ultrafilters obtained from Romicon with 10,000 Dalton pores. Next, the 10,000 Dalton ultrafilters are replaced with 50,000 Dalton ultrafilters also obtained from Romicon and the dialysis process is repeated. The dialysis process now separates the gel matrix polymers into two fractions; a first fraction consists of gel matrix polymers ranging in size from 10,000 to 50,000 Daltons and is discharged through separation discharge line 35, a second fraction consists of gel matrix polymers greater than 50,000 Daltons in size and is returned to vat I. This process can last from minutes to hours depending upon the amount of salt and water that is needed to be removed from a given product. This is important because topical aloe products must have the salt and water content adjusted if it is to be used on damaged skin. The salt content can cause burning and irritation.

In Figure 7, separation return line 36 is made of Tygon tubing (food grade) provided by the Texas Rubber Supply Company, Dallas, Texas. The separation discharge line 35 is a 316

stainless steel pipe distributed by Van Tone, Inc., Dallas, Texas.

EXAMPLE 2

Preparation of Aloe vera for drinking. By using 100 gallon (379 liters) of Aloe vera gel by Example 1, with the following specific preservatives:

EQUIPMENT REQUIRED:

1. Two (2) 100 gallon stainless steel tanks with mixer.
2. One (1) 1,000 gallon stainless steel tank with mixer.
3. Homogenizer with pump.
4. Stainless steel screen.
5. One (1) transfer pump of suitable capacity.
6. Connecting hoses and stainless steel fittings.

BLENDING PROCEDURE:

Prior to the addition of raw Aloe vera gel to the collection tank complete steps 1 and 2 below:

1. Add 40 gallons (151.4 liters) of D.I. Water
2. Dissolve, with agitation, the following chemicals in the deionized water in the collection tank (see item 13, infra):

   Sodium Benzoate
   Glycine
   Citric Acid
   Potassium Sorbate
   Vitamin E

3. With continued agitation, collect raw Aloe vera gel from the grinder into the collection tank to make a total volume of 100 gals. (379 liters).
4. Remove tank to the compounding area.
5. Place the next collection tank under the grinder discharge.
6. Set previously sanitized homogenizer at 1500 psi pressure and connect to the collection tank.
7. Start homogenizer and discharge product to open

stainless steel basket mounted in the 1,000 gallon stainless steel tank.

8. Start agitation when product covers mixers blade. Record time that agitation is started and finished, until 1,000 gal. tank is emptied (approx. 8 hours).
9. Continue to agitate, slow speed.
10. Dialyze product to adjust aloin content to 50 ppm or less and reduce excess acid if necessary.
11. Add vanilla flavor and cinnamon oil - natural W.S. and mix for twenty minutes.
12. Product is ready to fill. Fill immediately.

13. INGREDIENTS	AMOUNT REQUIRED
D.I. Water (40.0 gals.)	151.4 L.
Sodium Benzoate USP	378 g.
Glycine	3.0 kg.
Citric Acid USP	416 g.
Potassium Sorbate USP	189 g.
Vitamin E (1000 units)	1 capsule
Raw Aloe Vera Gel q.s. to (100 gals.)	379 L.
Flavor	
Vanilla	121.0 g.
Cinnamon Oil - Natural W.S.	8.0 ml.

EXAMPLE 3

Aloe vera gel for manufacturing Aloe vera cosmetics. One hundred gallons (379 liters) of fine homogenized Aloe gel prepared by Example 1 was pumped to a stainless steel diatomite filter made by Lomart of 960 Alabama Avenue, Brooklyn, NY. The filter was a diatomaceous earth filter typical of the type used in swimming pools. Substantially all fibrous material was removed from the homogenized aloe gel fillet - preservative solution by the diatomite filter to produce a pulp-free aloe gel fillet - preservative solution.

EQUIPMENT REQUIRED:

1. One (1) 100 gallon stainless steel tank with mixer.
2. One (1) 1,000 gallon stainless steel tank with mixer.
3. Homogenizer with pump.
4. Stainless steel screen.
5. One (1) transfer pump of suitable capacity.
6. Pool filter and attachments.
7. Connecting hoses and stainless steel fittings.

PRELIMINARY WORK:

1. Sanitize the previously cleaned tanks, mixers and fittings with 50% IPA solution and rinse free of IPA with hot deionized Water.
2. Drain 5% HTH solution from pumps and attached hoses. Flush with water.
3. Sanitize pump and attached hoses with 50% IPA solution. Flush pumps and attached hoses with hot deionized water until free of isopropyl alcohol (IPA).
4. Sanitize homogenizer and attached hoses and pumps with 50% isopropyl alcohol solution. Flush homogenizer and attached hoses with hot deionized water until free of IPA.
5. Sanitizing procedure for the pool filter:
   (A) Prepare a 5% dissolved solution of swimming pool chlorine HTH powder in water and circulate through the entire system for twenty minutes.

BLENDING PROCEDURE:

Prior to the addition of stabilized raw Aloe Vera gel to the collection tank complete steps 1 and 2.

1. Add 50 gals. (189 liters) of deionized water.
2. Dissolve, with agitation, the following chemicals in the deionized water in the collection tank:

Methylparaben

Potassium Sorbate

3. With continued agitation, collect stabilized raw Aloe vera gel from the grinder into the collection tank to make a total volume of 100 gals., (379 liters).
4. Remove tank to the compounding area.
5. Set previously sanitized homogenizer at 1500 psig pressure and connect to the collection tank.
6. Start homogenizer and discharge product to open stainless steel basket mounted in 1,000 gallon stainless steel tank.
7. Start agitation when product covers mixers blade. Record time agitation started.
8. Continue to agitate, slow speed for 20 minutes.
9. Add all 100 gallon portions manufactured to the 1,000 gallon tank.
10. Allow product to set over night.
11. <u>Preparation of pool filter</u>:
    A. Flush the sanitizing solution from the pool filter using hot deionized water.
    B. Add 1 kg. of Diatomaceous earth to 10 gallons of deionized water and mix until suspended.
    C. Circulate mixture through the pool filter until the water is clear.
12. Flush excess water from pool filter with product.
13. Circulate product through the filter until product is clear of pulp.
14. Mix for 30 minutes.
15. Dialyze, if necessary, to reduce yellow sap to 50 parts per million or less, and to keep osmolarity to less than 150 millosmole.

INGREDIENTS	AMOUNT REQUIRED
D. I. Water (50 gals.)	189 L.
Methylparaben	681 g.
Potassium Sorbate	379 g.
Raw Aloe Vera Gel q.s. to volume or to 100 gals.	379 L.

EXAMPLE 4

Production of Aloe Creme

Starting with 71.6 gallons (271 liters) of Aloe vera gel for manufacturing Aloe vera cosmetics prepared by Example 3.

EQUIPMENT REQUIRED:

1. One (1) suitable size round bottom, jacketed, stainless steel kettle equipped with counter-motion agitation and nylon scrapers.
2. One (1) suitable size cylindrical, jacketed, stainless steel tank equipped with a variable speed mixer.
3. One (1) transfer pump.
4. Tygon and/or polyethylene connecting hoses, stainless steel fittings, suitable size containers and stainless steel filter.

PRELIMINARY WORK:

Sanitize the previously cleaned kettle, tank, mixers, pump, hoses, and containers.

PREPARATION OF THE AQUEOUS PHASE:

1. Add the following items to the cylindrical tank and start agitation when Stabilized Aloe Vera Gel for Mfg. has covered the mixing blade:

   Stabilized Aloe Vera Gel for Mfg.

   Propylene Glycol

   Allantoin

Methylparaben

GERMALL 115® Imidazolidinyl urea (Sutton Laboratories, Chatham, NJ 07928)

Sorbitol 70

Phosphoric Acid

2. Heat to 75° C ± 5° C with agitation and maintain temperature.

3. Mix until all items are in solution.

PREPARATION OF THE OIL PHASE:

1. Into a separate kettle add the following items:

Glyceryl Stearate

Stearyl Stearate

Wickenol® 163 (Wickham Products, Inc., Huguenot, NY 12746)

Lexamine® P-13 (Inolex Chemical Co., Philadelphia, PA 19148)

Cetyl Alcohol

Lanolin

Mineral Oil (Light) (Kaydol) (Delta Solvents and Chemicals, Dallas, TX 75285)

Dimethicone

Propylparaben

Vitamin E Natural

LEXEIN® QX-3000 (Inolex Chemical Co., Philadelphia, PA 19148)

2. Heat to 75° C ± 5° C with slow agitation until material is melted and homogeneous.

FORMULATION OF EMULSION:

For successful emulsification, the oil and water phases should be added at the same temperature when blending. This process should then proceed without interruption.

1. With slow agitation, add water phase slowly to the

oil phase.

2. Immediately turn off steam, drain jacket and start force cooling with cold domestic water, continuing slow agitation.
3. q.s. to batch size with deionized water at 75° C ± 5° C if necessary.
4. When product cools to 40° C ± 5° C, add the fragrance.
5. When cooled to 30° C, stop agitation and submit sample for approval.

INGREDIENTS	AMOUNT REQUIRED
Stablized Aloe Vera Gel for Mfg. (71.6 gals.)	271 Kg.
Propylene Glycol	17.4 Kg.
Allantoin	908 g.
Methylparaben	690 g.
Imidazolidinyl Urea (Germall 115)	2.0 g.
Sorbitol 70	908 g.
Phosphoric Acid	1.05 Kg.
OIL PHASE	
Glyceryl Stearate (Lexemul® 515) (Inolex Chemical Co., Philadelphia, PA 19148)	35.6 Kg.
Stearyl Stearate (Lexol® SS) (Inolex Chemical Co., Philadelphia, PA 19148)	8.9 Kg.
Dioctyl Adipate (and) Octyl Stearate (and) Octyl Palmitate (Wickenol 163®, Wickam Products, Inc., Huguenot, NY 12746)	8.9 Kg.
Palmitamidopropyl Dimethylamine (Lexamine® P-13™, Inolex Chemical Co., Philadelphia, PA 19148)	3.5 Kg.
Cetyl Alcohol	3.5 Kg.

Lanolin	3.5 Kg.
Light Mineral Oil (Light) (Kaydol) (Delta Solvents and Chemicals, Dallas, TX 75285)	3.5 Kg.
Dimethicone	908 g.
Propylparaben	399 g.
Vitamin E Natural	363 g.
Cocamidopropyldimonium Hydroxypropylamino Collagen	363 g.
(LEXEIN® QX-3000 Inolex Chemical Co., Phiadelphia, PA 19148)	109 g.
Elias Fragrance #5394 (Elias Fragrances, Brooklyn, NY 11203)	109 g.

EXAMPLE 5

Preparation of a counter-irritant, starting with 115 gallons (436 Kg) of stabilized Aloe vera gel for manufacturing, prepared by Example 3.

EQUIPMENT REQUIRED:

1. One (1) suitable size round bottom, jacketed, stainless steel kettle equipped with counter-motion agitation and nylon scrapers.
2. One (1) suitable size cylindrical tank with a variable speed mixer.
3. One (1) transfer pump.
4. Tygon and/or polyethylene connecting hoses, stainless steel fittings, suitable size containers and stainless steel filter.

PRELIMINARY WORK:

Sanitize the previously cleaned kettle, tank, mixers, pump, hoses, and containers.

CARBOPOL SLURRY:

1. Add deionized Water to the mixing tank. Start mixer.
2. Slowly add all Carbomer 940 (Carbopol® 940) (B.F. Goodrich Chemical Group, Chicago, IL 60694) to the

mixing tank. Continue mixing until solvated.

3. Let set overnight. Turn mixer and mix for 1 hour.

PREPARATION OF THE AQUEOUS PHASE:

1. Add the following items to the cylindrical tank and start agitation when deionized Water has covered the mixing blade:

   Deionized Water (Retain 10 gals. to rinse Carbopol® Slurry from tank.)
   Stabilized Aloe Vera Gel
   Propylene Glycol
   Triethanolamine 99%
   Allantoin
   Methylparaben
   Potassium Sorbate
   Urea

2. Heat to 70° C ± 5° C with agitation and maintain temperature.
3. Mix until all items are in solution.
4. Add Carbopol Slurry and mix until free of "fish eyes".
5. Rinse tank with deionized Water.

PREPARATION OF THE OIL PHASE:

1. Into a kettle add the following items:

   Petrolatum (White Protopet®) (Delta Solvents and Chemicals, Dallas, TX 75285)

   Mineral Oil (Carnation White®) (Delta Solvents and Chemicals, Dallas, TX 75285)

   Mineral Oil (and) Lanolin Alcohol (Amerchol L-101) (Amerchal, Edison, NJ 08817)

   Acetylated Lanolin Alcohol (Fancol ALA®) (Delta Solvents and Chemicals, Dallas, TX 75285)

   Stearic Acid Triple Pressed

Glyceryl Stearate (Lexemul 515®) (Inolex Chemical Co., Philadelphia, PA 19148)

Cetyl Alcohol

Dioctyl Adipate (and) Octyl Stearate (and)

Octyl Palmitate (Wickenol® 163)

(Wicken Products Inc., Huegenot, NY 12746)

White Oleic Acid USP

Stearyl Stearate (Lexol® SS)(Inolex Chemical Co., Philadelphia, PA 19148)

Propylparaben

Butylparaben

Eucalyptus Oil

Methyl Salicylate

Camphor

Menthol

Histamine Dihydrochloride

Oil of Cinnamon

2. Heat to 70° C ± 5° C with slow agitation until material is melted and homogenous.

<u>FORMULATION OF EMULSION</u>:

Note: For successful emulsification, the oil and water phases should be added at the same temperature when blending. This process should then proceed without interruption.

1. With slow agitation, add water phase slowly to the oil phase.
2. Immediately turn off steam, drain jacket and start force cooling with cold domestic water, containing slow agitation.
3. q. s. to batch size with deionized Water at 70° C ± 5° C if necessary.

4. When cooled to 35° C, stop agitation and submit sample for approval.

INGREDIENTS	AMOUNT REQUIRED
Carbopol Slurry	
Deionized Water (30 gals.)	113 Kg.
Carbomer 940 (Carbopol® 940™) (B.F. Goodrich Chemical Group, Chicago, IL 60694)	4.36 Kg.
Water Phase	
Stabilized Aloe Vera Gel for Mfg. (115 gals.)	436 Kg.
Deionized Water (41.5 gals.)	157 Kg.
Propylene Glycol	54.5 Kg.
Triethanolamine 99%	9.8 Kg.
Allantoin	653 g.
Methylparaben	1.1 Kg.
Potassium Sorbate	545 g.
Urea	21.8 Kg.
Oil Phase	
Petroleum USP (White) (Delta) Solvents and Chemicals, Dallas, TX 75285)	8.2 Kg.
Mineral Oil (Carnation White) (Delta Solvents and Chemicals, Dallas, TX 75285)	8.2 Kg.
Mineral Oil (and) Lanolin Alcohol (Amerchol L-101™) (Americhol, Edison, NJ 08817)	7.6 Kg.
Acetylated Lanolin Alcohol (Fancol® ALA™) (Delta Solvents and Chemicals, Dallas, TX 75285)	7.6 Kg.
Stearic Acid Triple Pressed	23.5 Kg.

Glyceryl Stearate (Lexemul® 515) (Inolex Chemical Co., Philadelphia, PA 19148)	10.3 Kg.
Cetyl Alcohol	8.2 Kg.
Dioctyl Adipate (and) Octyl Stearate (and) Octyl Palmitate (Wickenol® 163) (Wicken Products, Inc., Huegenot, NY 12746)	10.3 Kg.
White Oleic Acid USP	1.1 Kg.
Stearyl Stearate (Lexol® SS) (Inolex Chemical Co., Philadelphia, PA 19148)	1.1 Kg.
Propylparaben	1.1 Kg.
Butylparaben	1.1 Kg.
Eucalyptus Oil	21.8 Kg.
Methyl Salicylate	136 Kg.
Camphor	21.8 Kg.
Menthol	21.8 Kg.
Histamine Dihydrochloride	545 g.
Oil of Cinnamon	218 g.

EXAMPLE 6

Preparation of freeze-dried Aloe. Starting with one liter of Aloe vera gel, prepared by Example 2. Place the juice in a freeze drying unit and produce approximately 45 grams of freeze-dried Aloe.

EXAMPLE 7

Preparation of a topical wound gel. Start with Aloe vera gel prepared by Example 3 and concentrate to 190 percent by dialysis.

EQUIPMENT REQUIRED:

1. Two (2) suitable size stainless steel tanks with mixer.
2. One (1) Romicon® concentrator system.
3. One (1) transfer pump.

4. Tygon® and/or polyethylene connecting hoses, stainless steel fittings, suitable size containers and stainless steel filter.

PRELIMINARY WORK:

1. Sanitize the previously cleaned tanks, mixers, pump, hoses, and containers used during the manufacturing process.
2. Prepare Carbopol® Slurry one day before manufacturing.
3. Sanitize the previously cleaned Romicon concentrator system. The entire system is washed with deionized Water and filled with 37% Hydrogen Peroxide and allowed to stand overnight.

CONCENTRATION OF ALOE VERA GEL FOR DERMAL WOUND GEL PREPARATION FOR TREATING DECUBITUS ULCERS:

1. Flush the concentrator system with deionized Water until free of Hydrogen Peroxide.
2. Separate the calculated quantity of effluent from the Aloe Vera Gel required to concentrate the product of 190% Gel.

CARBOPOL SLURRY:

1. Add Aloe Vera Gel - 190% to the mixing tank. Start mixer.
2. Slowly add the Carbomer® 940 (Carbopol® 940) (B.F. Goodrich Chemical Group, Chicago, IL 60694) to the water in the mixing tank. Continue mixing until solvated.
3. Let set overnight. Turn on mixer and mix for 1 hour.

MIXING PROCEDURE:

1. In a suitable size mixing tank, add the following items and start mixer when the blade is covered:

   Aloe Vera Gel 190%
   Panthenol
   Allantoin
   L-Glutamic Acid

Imidazolidinyl Urea (GERMALL® 115)
(Sutton Industries, Chatham, NJ 07928)
Potassium Sorbate
Sodium Benzoate
Triethanolamine 99%

2. Continue mixing while adding Carbopol® Slurry which has been strained through 100 mesh stainless steel screen.
3. Mix until free of "fish eyes".
4. Read the pH and record, pH range 5.0 ± 0.5.
5. Adjust pH if required with Citric Acid or Triethanolamine.
6. Record pH.
7. When product is complete, submit sample for approval.
8. When ready for filling, pump through 100 mesh stainless steel screen into filling hopper.

INGREDIENTS	AMOUNT REQUIRED
CARBOPOL® SLURRY:	
Conc. Stabilized Aloe Vera Gel 5X 190%) (75 gals.)	284 Kg.
Carbomer 940 (Carbopol® 940) (B.F. Goodrich Chemical Group, Chicago, IL 60694)	13.3 Kg.
Conc. Stabilized Aloe Vera Gel (5X 190%) (403 gals.)	1523 Kg.
Panthenol	37.9 Kg.
Allantoin	11.4 Kg.
L-Glutamic Acid	5.7 Kg.
Imidazolidinyl Urea (Germall® 115) (Sutton Laboratories, Chatham, NJ 07928)	1.9 Kg.
Triethanolamine 99%	13.3 Kg.
Potassium Sorbate	945 g.

Sodium Benzoate 1.9 Kg.

EXAMPLE 8

Starting with 100 kilograms of leaves straight from the field not washed. 51.2 kilograms of Aloe vera juice is collected using a "Thompson Aloe Juice Extractor" made by Thompson Manufacturing Co., Harlingen, Texas 78550. The extractor is operated according to the directions supplied by the manufacturer. The resulting 51.2 kilograms of Aloe juice extract has .1% methyl paraben added and mixed for 20 minutes in a one hundred gallon stainless steel tank with mixer. Then the solution is dialyzed using the Romicon® ultrafiltration equipment until the anthraquinone content is less than 50 ppm by weight and this gel is used to produce the Aloe Creme of Example 4.

SEPARATION AND ISOLATION PROCESS

As discussed above in the fractionation process, the filleted internal gel matrix portion of the Aloe vera leaf in the form of gel matrix strips can be homogenized and filtered to remove the interstitial fibers sub-portion, leaving the residual matrix sub-portion. The residual matrix sub-portion may then be treated to separate, isolate and purify the active chemical substance, Carrisyn®, in Aloe vera gel. To separate Carrisyn® from the residual matrix sub-portion, an excess of a water soluble, lower aliphatic polar solvent is added to the residual matrix sub-portion. Carrisyn® then begins to precipitate from this mixture. The solution is allowed to settle for a sufficient period of time to allow as much active ingredient to precipitate out of solution as possible but not so long that the Carrisyn® begins to degrade. After this period of time the supernatant is decanted or siphoned off without disturbing the settled precipitate. The precipitate and remaining solution is then placed in an appropriate centrifuge apparatus and is collected into a pellet. After centrifugation, the supernatants are decanted and discarded. Optionally, the pellets are washed with a fresh aliquot of the water soluble, lower-aliphatic polar solvent and collected again and the supernatants are again discarded. The pellet is then lyophilized to dryness and allowed to dry overnight. The resulting product is a substantially non-degradable

lyophilized form of Carrisyn®. The resulting product may be ground to form a powder.

An alternate and preferred process for separating and isolating Carrisyn® includes the following steps.

Leaves from mature, outdoor grown aloe vera plants are pulled or cut from near the base of the plant, preferably without breaking or damaging any part of the leaf prior to processing. The leaf is preferably cut at the base of the plant immediately above the stalk and is peeled from the stalk, in order to prevent leakage of the clear cellular gel or contamination of the gel with the yellow sap.

After removal from the plant, the butt and tip portions of the leaves are removed and the cut leaves are pared to form fillets as described above in the fractionation process.

The resultant fillet (internal gel matrix) is then ground, shredded or blended to break up the interstitial fibers present therein or the internal gel matrix may be forced through a wire mesh or filter screen in order to achieve liquefaction. The resultant liquefied internal gel matrix is then homogenized. The homogenized extract thus obtained typically has a pH of approximately about 4 to about 5, preferably about 4. The homogenized extract is then filtered to remove the interstitial fibers. The homogenized and filtered extract may then be treated in the identical manner as the residual matrix sub-portion, referred to immediately above, to separate and isolate Carrisyn®.

An additional alternate and preferred process for separating and isolating Carrisyn® includes the following steps.

Leaves from mature, outdoor grown aloe vera plants are pulled or cut from near the base of the plant, preferably without breaking or damaging any part of the leaf prior to processing. The leaf is preferably cut at the base of the plant immediately above the stalk and is peeled from the stalk, in order to prevent leakage of the clear cellular gel or contamination of the gel with the yellow sap.

The leaves may then be crushed by suitable means, for example a "Thompson Aloe Extruder" made by Thompson Manufacturing

Company, Harlingen, Texas, to extrude aloe juice. The extruded aloe juice may then be treated in the identical manner as the residual matrix sub-portion, referred to above, to separate and isolate Carrisyn®.

All steps in the processes described are performed at about room temperature except for the lyophilization step which is preferably performed at -50°C.

Various modifications of the disclosed processes and compositions of the invention, as well as alternative modifications, variations and equivalents will become apparent to persons skilled in the art upon reading the above general description. The following examples are illustrative only and are not intended to limit the scope of the appended claims, which cover any such modifications, equivalents or variations.

EXAMPLE 9

Process for Separating and Isolating Carrisyn®.

A. PRELIMINARY WORK:

1. Previously cleaned tanks, mixers and fittings were sanitized with 50% isopropyl alcohol (IPA) solution and rinsed free of IPA with hot deionized Water.

2. Pumps and attached hoses were drained with 5% "HTH" chlorine swimming pool solutions, then flushed with water.

3. The pumps and attached hoses were sanitized with 50% isopropyl alcohol solution. Pumps and attached hoses were flushed with hot deionized water until free of IPA.

4. An homogenizer and attached hoses and pumps were sanitized with 50% isopropyl alcohol solution. The homogenizer and attached hoses were flushed with hot deionized water until free of IPA.

Aloe barbadensis Miller leaves collected from the Rio Grande Valley were transferred in a refrigerated truck at 40 to 45°F within eight hours after harvest and stored under refrigeration at 40 to 45°F until processed to reduce degradation.

Twenty to sixty pounds of stored leaves were then placed in a prewash bath of an aqueous solution of calcium hypochlorite at room temperature substantially to remove surface dirt from the

leaves and kill surface bacteria on the leaves. The aqueous solution of calcium hypochlorite was prepared by adding approximately 0.125 grams of 98% calcium hypochlorite to one liter of water to produce a solution containing 50 ppm of free chlorine. The leaves remained in the prewash bath for a period of approximately five minutes.

Next, the substantially dirt and bacteria free leaves were placed on the horizontal conveyor belt of a "Thompson Aloe Washer" made by Thompson Manufacturing Company, Harlingen, Texas. The "Thompson Aloe Washer" washed the leaves with room temperature water to remove the surface dirt and aqueous solution of calcium hypochlorite from the leaves. Again, the leaves were visually inspected and hand scrubbed as necessary to remove any surface dirt remaining on the leaves. Such leaves were then rinsed with room temperature water.

The tip and butt portion was then removed from each leaf and the leaves were placed in stainless steel basket-type containers, placed together on top of a funnel shaped stainless steel collector, each container having a mesh bottom. Yellow sap was allowed to drain from the leaves for approximately 30 minutes. The yellow sap passed through the mesh bottom of the stainless steel basket and was collected in a funnel shaped collector.

The stainless steel basket type containers containing the aloe leaves were removed from the collector, and were then submerged in a second stainless steel vessel comprising a room temperature water bath of continuously horizontally flowing rinse water moving counter-current to the containers which are slowly moved by hand from one end of the vessel to the other, for approximately thirty minutes to one hour. This allows the yellow sap to drain further from the leaves. The leaves were allowed to soak in this solution for 30 minutes.

The leaves were then removed from this solution and the rind was removed from each leaf with a sharp knife or wire cheese slicer to produce an aloe gel fillet from each leaf portion. The aloe gel fillets were visually inspected and any contaminated

aloe gel fillets or fillet part detected by a characteristic yellowish discoloration were discarded. The total mass of uncontaminated aloe gel fillets was 20 to 60 percent the starting leaf mass depending on the leaf size and condition.

The uncontaminated aloe gel fillets were then placed in a 750 seat restaurant set stainless steel garbage disposal unit which coarse ground the fillets to an average particle size of the consistency of a thick, but freely flowing (coarse homogenized) liquid. The stainless steel garbage disposal unit was made by the N-sink-erator Division of Emerson Electric Co., Racine, WI, Model No. SS-150-13, Serial No. 115132.

The coarse ground aloe gel fillets then passed to a 100 gallon stainless steel holding vat. The holding vat was made by Process Equipment Corp. of Belding, Michigan, Model No. 100 gallon OVC, Serial No. 40865-3.

From the holding vat, the coarse ground aloe gel fillet solution was pumped to a homogenizer. The homogenizer was made by Crepaco Food Equipment and Refrigeration, Inc., of Chicago, Illinois, Serial No. 04-03. The homogenizer was of a type typically used in dairy processes for the homogenization of milk. The coarse ground aloe gel fillet solution was finely homogenized under a pressure of about 1,500 psi.

From the homogenizer the finely homogenized aloe gel fillet solution was pumped to a stainless steel storage tank. The storage tank was made by Process Equipment Corp. of Belding, Michigan, Model No. 1000 gallon OVC, Serial No. 40866-2. The total mass of the homogenized aloe gel fillet solution was 20 to 60 percent of the starting leaf mass. Then, if necessary, the homogenized product was dialyzed using ultrafiltration.

The finely homogenized aloe gel fillet solution was then filtered to remove the interstitial fibers using a Leslie's Diatomaceous Earth Filter Model DE-48. The interstitial fibers themselves instead of diatomaceous earth were used as the filter media, the fibers being supported by a nylon mesh cloth filter support. The gel was pumped through the filter for several minutes before opening the exit port so that a sufficient amount of

fibers could build up to serve as the filter media.

Twenty gallons of the filtered aloe gel fillet solution was then pumped into a 100 gallon tank and 80 gallons of 190 proof undenatured ethanol (Ethyl Alcohol, 190 proof, U.S.P., punctilious, 54 gal. Batch I.D. #CT185J04 available through U.S. Industrial Chemicals, Co., P.O. Box 218, Tuscola, Illinois 61953) was added to the aloe gel fillet solution.

The alcohol-gel solutions were then immediately transferred to several 11 quart pans 10½" diameter and 8" high of 18-8 stainless steel (Bloomfield Industries Inc., Chicago, Illinois, available through Watson Food Service Equipment and Supplies, 3712 Hagger Way, Dallas, Texas).

The alcohol-gel solutions were then allowed to settle for approximately four hours.

The clear liquid supernatant was then decanted or siphoned off using care not to disturb the precipitate that had settled on the bottom of the pans. The precipitate and remaining solutions were then transferred to four 1 pint stainless steel centrifuge buckets with about 500 g. of precipitate and remaining solution being transferred to each bucket. The buckets were then spun at 2000 x g for about 10 minutes using an IEC Centra-7 Centrifuge (International Equipment Co., 300 2nd Avenue, Needham Heights, Massachusetts 02194 available through: American Scientific Products, P.O. Box 1048, Grand Prairie, Texas 75050).

After centrifugation the supernatants were decanted and discarded. The pellets were then washed with fresh 190 proof, undenatured ethanol and collected again at 2000 x g for about 10 minutes. After spinning again the supernatant was discarded.

The pellet was then transferred to several 600 ml. VIRTIS lyophilization jars and swirled in liquid nitrogen until frozen. The lyophilization jars were then attached to a lyophilization apparatus consisting of a Welch Duo-seal Vacuum Pump (Model No. 1402, available from Sargeant-Welch, P.O. Box 35445, Dallas, Texas 75235), a Virtis Immersion Coil Cooler (Model No. 6205-4350 Cooler in acetone bath) and a Virtis 18 Port Vacuum Drum Manifold (Model No. 6211-0350). All Virtis equipment is

available from American Scientific Products, P.O. Box 1048, Grand Prairie, Texas 75050. The lyophilization drum was filled with acetone that was maintained at -50°C.

The samples were lyophilized to dryness overnight and were then weighed on a Mettler AE 163 balance. The samples remaining consisted of substantially non-degradable lyophilized Carrisyn®. The yield from 50 gallons of aloe vera gel was approximately 370-380 g. of Carrisyn®.

EXAMPLE 10

Process for Separating and Isolating Carrisyn®.

Aloe barbadensis Miller leaves collected from the Rio Grande Valley were transferred in a refrigerated truck at 40 to 45°F within eight hours after harvest and stored under refrigeration at 40 to 45°F until processed to reduce degradation.

The tip and butt portion was then removed from each leaf. The rind was then removed from each leaf with a sharp knife or wire cheese slicer to produce an aloe gel fillet from each leaf portion.

The aloe gel fillets were then placed in a 750 seat restaurant set stainless steel garbage disposal unit which coarse ground the fillets to an average particle size of the consistency of a thick, but freely flowing (coarse homogenized) liquid. The stainless steel garbage disposal unit was made by the N-sink-erator Division of Emerson Electric Co., Racine, WI, Model No. SS-150-13, Serial No. 115132.

The coarse ground aloe gel fillets then passed to a 100 gallon stainless steel holding vat. The holding vat was made by Process Equipment Corp. of Belding, Michigan, Model No. 100 gallon OVC, Serial No. 40865-3.

From the holding vat, the coarse ground aloe gel fillet solution was pumped to a homogenizer. The homogenizer was made by Crepaco Food Equipment and Refrigeration, Inc., of Chicago, Illinois, Serial No. 04-03. The homogenizer was of a type typically used in dairy processes for the homogenization of milk. The coarse ground aloe gel fillet solution was finely homogenized under a pressure of 1,500 psi.

From the homogenizer the finely homogenized aloe gel fillet solution was pumped to a stainless steel storage tank. The storage tank was made by Process Equipment Corp. of Belding, Michigan, Model No. 1000 gallon OVC, Serial No. 40866-2. The total mass of the homogenized aloe gel fillet solution was 20 to 60 percent of the starting leaf mass. Then, if necessary, the homogenized product was dialyzed using ultrafiltration.

The homogenized gel was then filtered to remove the interstitial fibers using a Leslie's Diatomaceous Earth Filter Model DE-48. The interstitial fibers themselves instead of diatomaceous earth were used as the filter media, the fibers being supported by a nylon mesh cloth filter support. The gel was then pumped through the filter for several minutes before opening the exit port so that a sufficient amount of fibers could build up to serve as the filter media.

Twenty gallons of the filtered gel was then pumped into a 100 gallon tank and 80 gallons of 190 proof undenatured ethanol (Ethyl Alcohol, 190 proof, U.S.P., punctilious, 54 gal. Batch I.D. #CT185J04 available through U.S. Industrial Chemicals, Co., P.O. Box 218, Tuscola, Illinois 61953) was added to the aloe gel fillet solution.

The alcohol-gel solutions were then immediately transferred to several 11 quart pans 10½" diameter and 8" high of 18-8 stainless steel (Bloomfield Industries Inc., Chicago, Illinois, available through Watson Food Service Equipment and Supplies, 3712 Hagger Way, Dallas, Texas).

The alcohol-gel solutions were then allowed to settle for approximately four hours.

The clear liquid supernatant was then decanted or siphoned off using care not to disturb the precipitate that had settled on the bottom of the pans. The precipitate and remaining solutions were then transferred to four 1 pint stainless steel centrifuge buckets with about 500 g. of precipitate and remaining solution being transferred to each bucket. The buckets were then spun at 2000 x g for about 10 minutes using an IEC Centra-7 Centrifuge (International Equipment Co., 300 2nd Avenue, Needham

Heights, Massachusetts 02194 available through: American Scientific Products, P.O. Box 1048, Grand Prairie, Texas 75050).

After centrifugation the supernatants were decanted and discarded. The pellets were then washed with fresh 190 proof, undenatured ethanol and spun down again at 2000 x g for 10 minutes. After spinning again the supernatant was discarded.

The pellet was then transferred to several 600 ml. VIRTIS lyophilization jars and swirled in liquid nitrogen until frozen. The lyophilization jars were then attached to a lyophilization apparatus consisting of a Welch Duo-seal Vacuum Pump (Model No. 1402, available from Sargeant-Welch, P.O. Box 35445, Dallas, Texas 75235), a Virtis Immersion Coil Cooler (Model No. 6205-4350 Cooler in acetone bath) and a Virtis 18 Port Vacuum Drum Manifold (Model No. 6211-0350). All Virtis equipment is available from American Scientific Products, P.O. Box 1048, Grand Prairie, Texas 75050. The lyophilization drum was filled with acetone that was maintained at -50°C.

The samples were allowed to dry overnight and were then weighed on a Mettler AE 163 balance. The samples remaining consisted of substantially non-degradable lyophilized Carrisyn®. The yield from 50 gallons of aloe vera gel was approximately 370-380 g. of Carrisyn®.

EXAMPLE 11

Standard Laboratory Scale Process for Separating and Isolating Carrisyn®

Approximately 50 pounds of Aloe barbadensis Miller leaves were washed with water and brushed to remove dirt, dried latex and other contaminants. The outer cuticle of each leaf was then removed and the whole fillets were placed in a large beaker (on ice).

In 1.5 liter batches a Waring blender was loaded with the whole Aloe fillets. The fillets were blended at high speed twice for two minutes at room temperature. The blended fillets were then cooled at 4°C to allow foam generated during blending to settle.

The blended Aloe juice was then filtered through four layers of cotton (Cleveland Cotton) to remove any fibrous cellulosic pulp. The filtrate was then passed through six layers of cotton and approximately 4 liters of Aloe juice was collected.

The Aloe juice was then placed in a large five gallon stainless steel container. To the filtered juice was added 16 liters of chilled ethanol (Fisher Ethanol reagent grade, Cat. No. A995). The ethanol was added slowly while stirring the Aloe juice. A flocculent precipitate formed and the mixture was stirred for 15 to 30 minutes and was allowed to settle at room temperature for about two hours.

The supernatant was then decanted off and the pellet was placed in a small blender to which one liter of deionized water was added. This mixture was blended for several minutes at low speed to wash the pellet and was then placed in an eight liter nalgene container. To this mixture was added four more liters of ethanol and the mixture was stirred for 30 minutes. The precipitant that formed was allowed to settle for about two hours.

The majority of the supernatant was then decanted off and the resultant pellet was centrifuged at 2000 x g for 20 minutes at room temperature to pellet the precipitant for easy decanting of the remaining solvent.

The pellet was then placed in a lyophilization flask and lyophilized overnight in a Virtis lyophilizer.

The lyophilized powder weighed 10.9 g. The percent yield was 0.273% or 2.73 x 10^{-3} g/ml.

CHARACTERIZATION OF CARRISYN®

From a historical perspective, Aloe vera has been used for centuries as a wound healer. It has also been thought to be good for gastrointestinal problems, including stomach ulcers, and to be beneficial in the treatment of arthritis. See for example, Duke, James A., CRC Handbook of Medicinal Herbs, CRC Press, Inc. Boca Raton, Florida, pp. 31, 1985; Henry, R., Cosmetics and Toiletries, An Updated Review of Aloe Vera: Allured Publishing Corp., Vol. 94, pp. 42-50, 1979; and Morton, Julia F., Folk Uses and Commercial Exploitation of Aloe Leaf Pulp: Economic Botany,

Vol. 15, pp. 311-316, 1961. As noted above, however, the chemical substance in Aloe vera responsible for these properties of Aloe vera has never been identified and characterized.

Using pharmaceutical screening techniques a polysaccharide extracted from Aloe vera has now been found to be the active chemical substance in Aloe vera. This polysaccharide will be hereinafter referred to as Carrisyn®. Carrisyn® is an ordered linear polymer of substantially acetylated mannose monomers. Other ingredients, such as proteins, organic acids, anthraquinones, vitamins and amino acids make up less than one percent of Carrisyn®. The concentration of Carrisyn® in Aloe vera has been found to be approximately 0.05 to 0.3 weight-percent of the Aloe vera juice. The yield or concentration of Carrisyn® in the leaves depends on leaf maturity.

Starting with the whole Aloe vera plant and using certain pharmacological models to screen for activity, the plant was systematically divided into parts. Each part was tested for pharmacological activity, and the inactive parts were discarded. Then the active part was divided into smaller and smaller parts each time discarding the inactive fractions and keeping the active fraction until the active substance, Carrisyn®, was isolated. The activity of the various fractions was measured using the stimulation of human fibroblasts in tissue culture and ulceroprotection in rats as pharmacological models.

The pharmacological data that evidences that Carrisyn® is the active chemical substance in Aloe vera can be summarized as follows:

1. The dose-response of Carrisyn® was the same as Aloe vera juice with an equivalent amount of Carrisyn®.

2. Carrisyn® was effective in the ulceroprotection model by different routes of administration namely intravenously, intraperitoneally and orally.

3. Carrisyn® accounted for 100 percent of the effects in both pharmacological models.

4. The chemical substance, glucomannan a substance similar to Carrisyn® from a completely different source, the

Konjac plant, provided some pharmacological response.

Carrisyn® has been shown in laboratory studies to increase up to 300% in 48 hours the replication of fibroblasts in tissue culture which are known to be responsible for healing burns, ulcers and other wounds of the skin and of the gastrointestinal lining.

Carrisyn® has also been shown to increase DNA synthesis in the nucleus of fibroblasts. The increase in DNA synthesis in turn increases the rate of metabolic activity and cell replication which are fundamental steps to the healing process.

Carrisyn® has been shown in controlled studies to increase the rate of healing in animals.

Carrisyn® has also been shown to be an effective treatment for gastric ulcers in animal studies. Over a three year period laboratory rats, the stomachs of which react similar to that of humans, were tested. Carrisyn® was found to be equivalent to or superior to current medications used for the treatment of gastric ulcers. Most such products act to inhibit hydrochloric acid in the stomach. Carrisyn® works on a different principle and does not alter the natural flow of digestive acids.

As noted above, Carrisyn® can be precipitated out of liquidified *Aloe vera* gel by the addition of a water soluble, lower aliphatic polar solvent, preferably ethyl alcohol. Carrisyn® powder can then be prepared by lyophilization and optionally the lyophilization product can be ground into a powder with a grinding apparatus such as a Moulinex coffee-grinder (available from Dillard's, Dallas, Texas). Carrisyn® powder is highly electrostatic and is an off-white to pinkish-purplish amorphous powder depending on the oxidization state of any anthraquinone contaminant. Carrisyn® is stabilized and becomes substantially non-degradable by freeze drying or lyophilization which removes the water which causes hydrolysis. Freeze dried *Aloe vera* gel with a given amount of Carrisyn® has maintained its effectiveness for two years. It is believed that Carrisyn® in freeze dried form will be stable for up to ten years.

Heat and time have been found to be important factors in

the production of powdered Carrisyn®. Heat can aid in the hydrolysis or degradation of Carrisyn® and the longer it takes to process out the Carrisyn® at a given temperature, the more is the degradation. Accordingly, it is preferred that the process which allows for the quickest extraction of Carrisyn® from whole Aloe vera leaves be used when high molecular weight Carrisyn® powder is desired and it is preferred that the process which allows for the slowest extraction of Carrisyn® from whole _Aloe vera_ leaves be used when low molecular weight Carrisyn® powder is desired.

Rehydration of Carrisyn® powder at a weight/volume concentration of 0.2 to 1 percent resulted in the reformation of a viscous "gel" like fresh _Aloe vera_. The gel-like consistency which returns upon rehydration of Carrisyn® powder is indicative of the high molecular weight polysaccharidic nature of Carrisyn®. Generally, as polysaccharides are degraded or hydrolyzed their viscosity is lowered. Accordingly, the viscosity of rehydrated Carrisyn® powder gives a good indication of quality and may be used as a parameter for quality assurance.

Carrisyn® produced according to the process of the present invention may be characterized as a substantially non-degradable lyophilized ordered linear polymer of acetylated mannose monomers, preferably bonded together by β $(1 \rightarrow 4)$ bonds.

Various modifications of the disclosed compositions of the invention, as well as alternative modifications, variations and equivalents will become apparent to persons skilled in the art upon reading the above general description. The following Examples (Examples 12-44) were conducted in order to further characterize and identify Carrisyn® and similar polysaccharides isolated from other Aloe species. The following examples are illustrative only and are not intended to limit the scope of the appended claims, which cover any such modifications, equivalents or variations.

EXAMPLE 12

Comparative High Performance Liquid Chromatography (HPLC).

A commercially available glucomannan (a polysaccharide

isolated from the Japanese Konjac plant, purchased from General Nutrition Centers) showed some similar behavior as Carrisyn® in the pharmacological screening models. This Konjac mannan (henceforth called glucomannan) though utilized to aid in the characterization of the Carrisyn® was found to be considerably different.

Initial experiments were done on the glucomannan and the Carrisyn® in parallel. Both of these polysaccharides were subjected to acid hydrolysis in 1 molar (M) sulfuric acid (.01 grams (g) polysaccharide/2 milliliters (ml) sulfuric acid) for 24 hours in vacuo at 110 degrees celsius. High Performance Liquid Chromatography (HPLC) of the hydrolyzed products (Bio Rad Aminex HPX-87C Column; R.I. detector; flow rate 0.6 ml/min.; eluting solvent, HPLC grade water) showed mannose present and a peak that coeluted with the glucose standard. The "glucose" peak was a wide, skewed peak which indicated that it may contain modified glucose. Acid hydrolysis at slightly different concentrations gave similar results with the glucomannan yielding definite glucose and mannose products and the Carrisyn® yielding a definitive mannose peak and a peak that at least migrates with a similar retention time as the glucose standard.

The two polysaccharides were then subjected to enzymatic hydrolysis with a crude cellulase preparation. When the commercial glucomannan was hydrolyzed and analyzed by HPLC (same conditions) well resolved glucose and mannose peaks and three other peaks which migrate faster than glucose were seen. These peaks indicative of di-, tri- and tetrasaccharide moieties, were possibly due to branching of the polysaccharide or incomplete hydrolysis. Since the enzyme preparation was a semi-crude preparation, other sugar hydrolases were no doubt present and an accurate determination of the enzyme preparation's bond specificity could not be made. When Carrisyn® was subjected to the same enzymatic treatment, a very different profile was seen on HPLC. The enzymatic hydrolysis yielded mannose, glucose and at least two larger peaks corresponding to larger, unhydrolyzed oligomers. In fact, most of the polysaccharide could be found in these

larger molecular fragments. This appears to be an indication that the structure (i.e., type of bonding and degree of branching) may be significantly different from the commercial glucomannan. Acid hydrolysis is a general hydrolysis method and enzymatic hydrolysis is much more specific as to the type of linkage it acts upon.

EXAMPLE 13

To aid in the characterization of Carrisyn®, the large polymeric saccharides were broken down through chemical hydrolysis. Hydrolysis was performed in 2N Trifluoroacetic acid solution (2N TFA) adapted from the technique developed by Albersheim, P. et al (Albersheim, P.; Nevins, D.J.; English, P.D.; and Karr, A (1967) Carbohydr. Res. 5, 340-345). The hydrolysates were vacuum oven dried and reconstituted in 0.01N H_2SO_4 for HPLC separation.

HPLC Separation of the Hydrolysates:

Separation of the hydrolysates was performed on a Hewlett Packard Liquid Chromatograph Model 1084-B. A Biorad model 1770 refractive index detector was attached to the chromatograph to monitor the effluent. The separating column was an Interaction ORH-801 organic acid column which contained a 0.65 x 30cm bed packed with an Interaction cation - exchange resin in hydrogen form.

The mobile phase was 0.01N H_2SO_4 at a flow rate of 0.5ml/min at a temperature of 35°C. Monomeric sugars and acid standards were used to establish retention times of the chromatograms. Based on these conditions, the retention times of galacturonic acid, glucose, mannose, galactose, rhamnose and arabinose were established approximately as 7.45, 8.04, 8.69, 8.86, 9.20 and 9.78 minutes respectively.

When the hydrolysates of Carrisyn® samples were separated under identical conditions, some of the sugars were identified in various amounts as shown below:

Table 1

Sample #	Galacturonic (Area)	Glucose (Area)	Mannose (Area)	Rham nose	Arabi nose (Area)	Acetic acid (Area)
Batch #1	Trace	476	14279	-	-	5818
Batch #3	584	Trace	16926	-	-	4538
Batch #3B	2329	Trace	15068	-	-	5553
Batch #5B	592	209	13158	-	-	7888
Batch #8B	1236	Trace	15049	-	-	5392
Batch #6B	Trace	329	12686	-	-	7465
C-701008	Trace	Trace	13734	-	-	6240
Batch #4	Trace	1260	17800	-	535	7699
Batch 8B (Dialyz)	Trace	286	13715	-	-	6677
C-613006	Trace	Trace	13571	-	-	5782
*Sucrose Octa acetate	-	2580	-	-	-	6407
*Glucomannan (Konjac)	441	10380	15050	-	-	5553

(*Glucommannan from the Konjac plant and sucrose octaacetate were included as reference standards.)

The liquid chromatographic separation of the hydrolysates demonstrates that Carrisyn® is essentially mannose. There are also varying amounts of galacturonic acid, glucose, rhamnose, arabinose and other sugars. Some of these sugars are difficult to determine accurately by this technique because of their low amount in the solution. The presence of acetyl groups as acetic acid, though in large amounts could not be completely assigned to Carrisyn® because the membrane filter used to clarify the hydrolysate solution appeared to add varying amounts of acetates into the chromatogram. It is noted that glucose is significantly very small in some Carrisyn® samples analyzed by this hydrolysis method. However, when Carrisyn® is hydrolyzed in mineral acids,

such as H_2SO_4, the glucose content is much higher perhaps due to the hydrolysis of the cellulosic materials present in the extract.

The observation that Carrisyn® is largely mannose was also confirmed by gas chromatography/mass spectrometry (GC/MS) separation and identification of the trimethylsilane (TMS) derivatives of the hydrolysate monomers. These techniques demonstrated that mannose accounted for more than 80% of the hydrolysis product.

EXAMPLE 14

Optical Rotation:

Carrisyn® was found to be levorotatory on the sodium D-line at 589 nanometers. A 1% (w/v) Carrisyn® solution in 25% methylmorpholine oxide in dimethylsulfoxide solvent gave a specific rotation at room temperature of (-) 26°. The value was measured against 25% MMNO in DMSO as the blank. The polarimeter used was Perkin Elmer 241 MC.

The optical rotation of aqueous solutions of Carrisyn® samples was determined using a Perkin-Elmer polarimeter Model 241MC. The measurements were made on a sodium line wavelength of 589 nm. The sample cell was of one decimeter (dm) pathlength.

Method:

Carrisyn® samples totaling 200 mg were weighed accurately and placed into a 200 ml polypropylene bottle. Deionized water (18.6 MΩ, 100 ml) was added to give a concentration of 0.2% (w/v). The suspension was allowed to agitate for 12 hours in a Lab-Line Orbit Shaker to effect a complete dissolution (commercial glucomannan from the Konjac plant was partially dissolved).

A typical solution of Carrisyn®, which is usually cloudy and viscous was filtered through a 1.2 μm membrane filter (Uniflow #46-02360, Schliecher and Schnell Inc., Keene, NH). The filtrate was refiltered through a 0.45 μm filter (Uniflow #46-02340 to a clear solution. The optical rotation was measured with deionized water (18.6 MΩ) as a blank. The measured rotation angle was converted to the specific rotation as follows:

$$\alpha = [\alpha]^{t}_{\lambda} g/v$$

$$[\alpha]^{t}_{\lambda} = v/dg$$

where $[\alpha]^{t}_{\lambda}$ = specific rotation

α = measured rotational angle

v = volume of solution (ml)

g = weight (g.) of active substance in v ml of solution

t = temperature (25 °C)

λ = wavelength (589 nm)

The results are shown in Table 2.

Table 2

Batch #	Specific Rotation + S.D.
# 1	-25.9 ± 0.6
# 3	-10.2 ± 0.5
# 4	-21.4 ± 0.5
# 613006	-14.7 ± 0.8
# C701008	-12.7 ± 0.4
# 3B	-14.2 ± 0.7
# 5B	- 9.5 ± 0.2
Glucomannan	-19.0 ± 0.4

The optical rotation demonstrates that Carrisyn® is levorotatory. The degree of rotation may vary from batch to batch.

All of the above data indicate that Carrisyn® is a polymer having a β (1 → 4) bond linkage.

EXAMPLE 15

Elemental Analysis:

Elemental analysis of organic compounds is a powerful tool in structure elucidation. Carrisyn® is substantially organic in nature and therefore amenable to this technique. Sample batches of Carrisyn® were sent to Huffman Laboratories Incorporated in Wheat Ridge, Colorado for elemental analyses. Oxygen, carbon, hydrogen, and nitrogen accounted for 80-90% of the make up of Carrisyn®. Phosphorus and sulfur together accounted for 0.5-2%. Table 3 illustrates the reported results.

Table 3

Elements %

Sample #	Carbon	Hydrogen	Oxygen	Nitrogen	Sulfur	Phosphorus
Batch #1	37.62	5.71	45.85	1.27	0.31	0.61
Batch #2	30.33	4.67	45.16	1.15	0.34	0.74
Batch #3	29.58	4.23	44.30	0.46	0.28	0.33
Batch #4	39.86	5.84	46.43	0.52	0.16	0.60
Batch #5	37.45	5.76	46.29	0.81	0.18	0.68
Batch #6	41.72	6.11	46.14	0.84	<.30	0.48
Batch #7	39.20	5.66	45.40	1.36	<.30	0.54
Hilltop	36.45	5.35	44.07	1.51	0.44	1.02
Mithcell	41.57	5.96	43.25	1.35	0.37	0.71
TCX	33.47	5.03	44.53	1.57	0.46	1.25

It is significant that for batches #3, #4, #5 and #6, the nitrogen content was comparatively low. These batches were prepared from denatured alcohol. Their physical appearance was also different from the majority of the Carrisyn® samples. Moreover, the cell culture responses (human fibroblast stimulation) of these batches were comparatively lower than the average.

EXAMPLE 16

Emission Spectrographic Analysis:

Emission spectrographic analysis was performed on Carrisyn® samples. The analysis was performed by Huffman Laboratories Inc. in Wheat Ridge, Colorado. A total of 68 elements from silver (Ag) to zinc (Zn) was monitored. Only a few of the elements were detected. These were calcium, sodium, silicon, magnesium, manganese, copper, chromium, barium, iron, and aluminum. Environmental Protection Agency (EPA) listed toxic metals such as lead, molybdenum, cobalt, cadmium, arsenic, selenium,

mercury among others were not detected by this technique which is semiquantitative.

EXAMPLE 17

X-ray diffraction Analysis:

The powder X-Ray diffraction pattern of Carrisyn® was obtained using a Philip Electronic Instrument X-Ray diffraction unit. A range of 5° - 60° [degrees] two-theta (2 θ) angle was scanned at the rate of (2 θ) angle per minute. The radiation was Copper K α (CuKα) from a tube operated at 15 milliamperes and 35 kilovolts.

Most Carrisyn® samples examined by X-ray diffraction analysis demonstrated no appreciable crystallinity. Carrisyn® was essentially amorphous. The amorphous nature of Carrisyn® can be destroyed by deacetylation and slightly by air drying of a rehydrated form.

EXAMPLE 18

Thermogravimetric Analysis:

This technique measures change in loss of weight with temperature. Both laboratory prepared and large scale manufactured Carrisyn® samples were analyzed by using a Mettler Thermogravimetric Analyzer TA 3000 system. The system was composed of a TC 10A TA processor and a TG 50 thermobalance. The temperature range was ambient [25°C] to 750°C.

Carrisyn® demonstrated a simple decomposition pattern inferring a possible simple structure. The decomposition profile differed from that of glucomannan from the Konjac plant. Carrisyn® lost surface and bound water between 50°C-210°C with the main peak at 95°C. The bulk of the decomposition took place between 210°-405°C with a peak near 325°C. More decompositions occurred between 406°C-735°C. The oxidizable carbons were eliminated from 630°C-737°C. The residue of largely ash accounted for more than 10-20% of the total weight. Table 4 illustrates the thermogravimetric decomposition profile of several samples of Carrisyn®.

Table 4

Sample #	Sample (mg)	H_2O %	Decompositions % Total wt.			Oxidizable %	Residue %
			1	2	3		
606005	6.53	9.35	49.66	11.59	6.03	16.4	7.70
711009	5.60	6.55	40.08	11.49	5.50	19.09	18.74
430003	8.24	9.33	36.43	10.76	4.60	24.69	18.65
620007	10.17	10.25	38.21	10.27	3.62	25.68	17.83
701008	5.79	9.65	45.10	10.55	5.09	13.43	17.25
531004	4.96	8.92	48.94	11.14	6.38	11.98	13.98
613006	4.86	8.16	46.3	10.75	5.40	15.41	16.38
Mex. 4 ply	8.03	8.46	26.04	11.55	3.46	31.61	25.84
Man. Batch 1		9.22	58.2	12.70	4.70	3.52	10.74
Man. Batch 2 Acetyl.	7.96	12.81	37.63	10.16	6.84	15.28	18.97
531004	7.74	5.45	66.94	6.89	3.69	15.0	2.51
Glucomannan from Konjac	30.6	9.82	11.32	60.83	0	17.4	0.668

It was observed that the decomposition profile was nearly the same irrespective of the source of the aloe leaves from which the Carrisyn® sample was made. The profile was significantly different from glucomannan of the Konjac plant. The manufactured batch #1 did portray some differences. This was not surprising because it was produced by a markedly different process. The precursor gel was filtered clear through a diatomaceous earth filter before Carrisyn® was precipitated from the gel with alcohol.

EXAMPLE 19

Density:

The density of Carrisyn® may vary depending on the amount of inorganic salts which co-precipitated with the Carrisyn®.

The density of Carrisyn® is also highly dependent on hydration. By adding additional water to the product prior to lyophilization, a fluffier, less dense product is produced. To maintain consistency the following samples were produced by a standard method using a ratio of 4:1 ethanol to water v/v during their manufacture, prior to freeze-drying.

Procedure:

Dry Carrisyn® powder was added to a preweighed 10 ml graduated cylinder, then the cylinder was weighed again to determine the total amount of Carrisyn® added. All weights were measured on a Mettler AE 163 analytical balance. The cylinder was then agitated on a Vortex-Genie agitator for 10 seconds on the highest speed, then the volume of Carrisyn® was observed. The density was determined as mass (grams) per volume (milliliters) of the dry Carrisyn® powder.

The results are shown in Table 5.

Table 5

Batch	Density
# 1	0.074
# 3	0.640
# 4	0.382
# 613006	0.158
# 701008	0.291
# 3B	0.561
# 5B	0.759
# 6B	0.626
# 7B	0.683
# 8B	0.499
Glucomannan	0.596

One gram of dry Carrisyn® powder from Batch #5B was dissolved in 50 ml of deionized water. The solution was then lyophilized. The dry Carrisyn® was a fluffy white powder having a density of 0.110. Thus, by redissolving the Carrisyn® from Batch #5B in 50 ml. of water and then lyophilizing the solution, the density of the dry powder went from 0.759 to 0.110.

EXAMPLE 20

SOLUBILITY OF CARRISYN®:

Carrisyn® demonstrates varying solubility characteristics which depend largely on the source of the aloe leaf, the degree of processing such as filtration, ethanol precipitation and drying. Degrees of aqueous solubilities of different batches of Carrisyn® have been observed. The same concentration (wt/volume) of different Carrisyn® batches may produce aqueous solutions (sols) very much different in viscosities and inorganic salt content. Viscosity and inorganic salt content generally affect the extent of solubilization of a material.

The present study was done with Carrisyn® Manufactured Batch #1 and Batch #2. The two batches have some differences in both source, processing and some chemical composition Solubilities in water and other media may differ. The solvents used were water, acetone, dimethylsulfoxide (DMSO), Tetrahydrofuran (THF), 0.9% sodium chloride and 0.1% sodium benezoate.

Method:

A total of 18 polypropylene tubes were set up. Six for each Carrisyn® batch and the remaining six tubes were for solvent blanks.

Into each of the sample tubes, 0.04 grams of Carrisyn® was weighed and added. Ten milliliters (10 ml.) of the solvent was added producing a 0.4% (wt./volume) mixture. The mixture was subjected to agitation for 5 hours at room temperature. The suspension was transferred to centrifuge tubes and centrifuged for 50 minutes at 1,500 rpm. The solvent blanks were similarly treated.

The solutions were decanted from the solids. Both the solutions and the solids (deposits) were dried in an oven at low heat <50°C until completely dried. The dried solids were weighed. The solvent flasks that showed deposits were also weighed. This weight was subtracted from the weight of the samples.

Table 6

Solvent	Batch No.	Decanted Solution (gm/100ml)	Deposit gm/ (100ml)	Total gm/ (100ml)
water(H_2O)	1	0.338	0.080	0.418
	2	0.384	0.035	0.419
acetone	1	0.0	0.365	0.365
	2	0.0	0.353	0.353
dimethylsulphoxide	1	0.282	0.125	0.407
	2	0.202	0.208	0.410
tetrahydrofuran	1	0.04	0.358	0.398
	2	0.04	0.351	0.391
0.9% sodium chloride	1	0.376	0.041	0.417
	2	0.418	0.019	0.437
0.1% sodium benzoate	1	0.354	0.083	0.437
	2	0.342	0.026	0.368

Results:

Carrisyn® was more soluble in water and aqueous solutions than any other solvent tested.

The concentration 0.4% (wt./volume) was chosen as a compromise between high viscosity in aqueous medium and very low solubility of Carrisyn® in other solvents. It is possible to get more Carrisyn® dissolved in water to about 0.5% (w/v).

EXAMPLE 21

Viscosity:

Viscosity measurements were made using a Cannon-Fenske type viscometer (Model 150, Industrial Research Glassware Ltd., Union, NJ) at 40°C. Aqueous Carrisyn® 0.2% solutions (0.05% NaN_3) were loaded into a viscometer and allowed to warm for ten minutes in a water bath (Model SBG-1090, Blue M Electric Co., Blue Island, IL). The solution flow was then observed to the nearest 0.1 second according to the manufacturer's directions. The calibration factor was 0.04197 centistokes/sec. at 40°C.

The results are shown in Table 7.

Table 7

Batch #	Viscosity (CentiStokes)
# 1	6.10
# 3	1.18
# 4	5.09
# 613006	2.12
# C701008	2.08
# 3B	2.02
# 5B	4.53
# 6B	1.75
# 7B	1.55
# 8B	1.97

EXAMPLE 22

Calcium Concentrations:

Although the function of calcium in Aloe vera activities is not completely established, the quality of Aloe vera gel is at times based on the calcium and magnesium content. Since Carrisyn® is the active substance in Aloe vera gel, the calcium content was monitored.

The total calcium in Carrisyn® was determined spectrophotometrically using a Calcium Rapid Stat Kit (Lancer Medical, St. Louis, MO). 50 μ l samples of aqueous 0.2% Carrisyn® solutions (0.05% NaN_3) were added to 3.0 ml of reagent, and the blue calcium-methylthymol complex was observed at 612 nm using an IBM Model 9420 UV-Visible Spectrophotometer.

Bound calcium was determined by the following empirical procedure:
25 ml samples of aqueous 0.2% Carrisyn® solutions (0.05% NaN_3) were placed in dialysis bags and dialyzed against 10 liters of 18.6 MΩ deionized water overnight at room temperature. The contents of the bags were then transferred to lyophilization jars and freeze-dried. The dry powders were weighed, then digested overnight in 20% nitric acid, 10% hydrochloric acid solution at 80°C. The samples were sent to Trac Laboratories in Denton, Texas for the determination of calcium by atomic absorption spectroscopy. Note: All containers in the above procedure were made of Nalgene or polypropylene, and had been extensively washed with 1.0 M HCl and deionized water (18.6MΩ).

The results are shown in Table 8.

Table 8

Batch	Total Calcium % (Dry Carrisyn®)	Bound Calcium % (Dry Carrisyn®)	Weight (mg) of lyophilized solids
# 1	3.7	0.042	31.7
# 3	10.15	0.066	12.9
# 4	2.15	0.048	32.0
# 613006	6.85	0.060	20.6
# C701008	6.65	0.068	23.9
# 3B	6.50	0.074	20.7
# 5B	3.55	0.074	29.6
Glucommannan	--------------negligible--------------------		

EXAMPLE 23

Nuclear Magnetic Resonance Spectroscopy (NMR):

NMR is a powerful technique that is indispensable in molecular structure elucidation. NMR spectra are calibrated using reference standards (tetramethylsilane, etc.) for chemical shift. Because of the inherent low solubility and high viscosity of Carrisyn® in some solvents generally used in NMR spectroscopy (D_2O, DMSO, Acetone-D, etc.) the technique has not been successful in Carrisyn® solutions.

However, excellent spectra of Carrisyn® as a solid, were obtained with a Cross Polarization - Magic Angle Spinning (CP-MAS) technique. A solid phase Carrisyn® C-13 NMR spectrum was obtained. The spectrum was produced by an IBM Spectrometer, with some special accessories attached to the IBM NMR spectrometer. Based on the spectrum, the C-13 NMR peaks of Carrisyn® analyzed were located at 20.54, 60.39, 71.33, 100.75, 171.78 and 180.75 parts per million (ppm) chemical shift with tetramethylsilane (TMS) as an external reference standard.

Tentatively, these peaks are assigned as follows: 20.54 ppm (<u>C</u>H3COH or <u>C</u>H3CO2); 60.39 (<u>C</u>H3O, <u>C</u>H2OH); 71.35 for C-2, C-3, C-4 and C-5 carbons. This assignment is perhaps correct in view of the size of this particular peak. The peak at 100.75 ppm may be due to (C-1 glycosidic). The 171.78 peak is slightly lower than is expected for <u>C</u>OOH but may be dependent on the orientation

of the molecule. The last peak at 180.75 ppm is a carbonyl carbon (C=O). Some small peaks about 95 ppm corresponding to C-1 (reducing end) were also observed. Significantly absent are any peaks between 106-109 ppm which may indicate C-1 of furanosyl unit.

These assignments are consistent with the structure of a β (1 → 4) mannan.

EXAMPLE 24

Infrared Spectroscopy: Infrared spectroscopy has been effective in the identification and characterization of Carrisyn®. An IBM IR/32 Fourier Transform Infrared spectrometer was used for this technique. The instrument is self calibrating to wave length by software and a He-Ne Laser, which can be verified by a standard spectrum of polystyrene film. Optical alignment is occasionally required when the signal to noise ratio decreases.

Carrisyn® is ground to a powder and mixed with an infrared grade potassium bromide [KBr] powder. The mixture may be pressed into a transparent disc which is scanned over infrared light. A new technique has been developed of making transparent films of 0.2% [w/v] aqueous Carrisyn® which demonstrate better spectrum resolution than KBr discs. When Carrisyn was scanned from 4000-400 cm^{-1}, the following functional groups were observed in the spectrum: 3600-3200 cm^{-1} due to the stretching frequency [O-H] hydrogen bonded and the related 1100-1050 cm^{-1} bending vibration. The stretching vibration at 2950-2800 cm^{-1} and the corresponding bending frequency 1470-1460 cm^{-1} were C-H groups. The other observed major functional groups were:

[1] The COO^- unit whose assymetric and symmetric vibrations were located between 1600-1550 cm^{-1} and 1450-1400 cm^{-1}, respectively.

[2] The ester groups [COOR] whose stretching vibration frequencies were observed between 1746-1735 cm^{-1} and 1250-1240 cm^{-1}.

[3] The amide groups [CONH] whose Amide I stretch and Amide II bending vibration were located near 1650-1645 and

1550-1540 cm^{-1}, respectively. Table 9 is included here to illustrate approximate frequencies [wave numbers], the corresponding functional group and the vibration mode.

Table 9

Peak #	Wavenumber(cm^{-1})	Functional Group	Vibrational Mode
1	1100-1035	O-H, C-O-C	v (O-H)
2	3430-3390	O-H	v (O-H)
3	2930-2800	C-H	v (C-H)
4	1470-1380	C-H	δ (C-H)
5	1600-1550	COO^- , $-C(=O)O^-$	v (C=O) assy.
6	1450-1400		v (C=O) sym
7	1420-1410	C-O	
8	1650-1645	CONH	v Amide I C=O
9	1550-1540	CONH	v Amide II
10	1746-1735	COOR	v (C=O)
11	1250-1240	COOR	v (C-O-C)
12	980-950	C-O, O-CH_2	

Table 10 is presented below to demonstrate how the peak absorption maximum of various Carrisyn® samples match Table 9 peak assignments.

Table 10

Carrisyn® 531004 [cm^{-1}]	Carrisyn® Hilltop [cm^{-1}]	Carrisyn® Batch #2 [cm^{-1}]
1591.5	1068.7	1585.7
3414.4	1037.8	3400.9
1072.6	3420.2	1086.1
1246.2	1244.2	1425.6
1740.0	1734.2	1037.8
1431.4	1635.8	1244.2
603.8	1653.2	1740.0
2930.2	1375.4	605.7
806.3	472.6	679.0
	1419.8	2928.3
	1558.7	545.9
	605.7	956.8
	2924.4	806.3
		879.6

Based on the infrared analysis, Carrisyn® is perhaps a polydisperse heteropolysaccharide with some acid, ester

[O-acyl/N-Acyl] functional group side chains. Carrisyn® samples have been fully O-acetylated and also deacetylated. The carbonyl functional group and the C-O-C stretches of acetylated Carrisyn® samples were observed between 1748-1735 cm^{-1} and 1246-1235 cm^{-1}, respectively. The carboxylate carbonyl stretches of deacetylated Carrisyn® sample were located between 1600-1550 cm^{-1} and 1450-1400 cm^{-1}, respectively as follows:

Table 11

Carrisyn® 531004 cm^{-1}	Carrisyn® 531004 [Deacetylated] cm^{-1}	Carrisyn® 531004 [Acetylated] cm^{-1}
1591.5	1431.4 [1560-1339]	1244.2
3414.4	3400.9	1741.9
1072.6	1032.0	1064.8
1246.2	1062.9	3460.7
1740.0	1091.8	1375.4
1431.4	875.8	1647.4
603.8	1147.8	1433.3
2930.2	1645.5	2932.2
806.3	2924.4	1541.3
	814.1	956.8
	615.4	603.8

When Carrisyn® was deacetylated, the absence of the ester C-O-C stretch frequency between 1248-1235 cm^{-1} was observed. It was also noticed that the largest peak of the spectrum was centered at 1431 cm^{-1}. The degree of acetylation/deacetylation of Carrisyn may be monitored by these two peaks. Actually, for this particular example, the peak at 1431 cm^{-1} [1560-1339cm^{-1}] was contaminated by an amide II peak since amide I could be observed at about 1646 cm^{-1}.

EXAMPLE 25

Biological Activity:

It has been observed by both in vivo and in vitro experiments that Carrisyn® promotes the proliferation of fibroblast cells. Promotion by a factor of 2-3 over the control has occasionally been recorded. Table 12 illustrates fibroblast proliferation counts influenced by Carrisyn® at a concentration of 0.1% (w/v) over a 72 hour period.

Table 12

Sample #	Conc.% (w/v)	24 hrs. %	48 hrs. %	72 hrs. %
TCX Lab. 7/25/85	0.1	90.7	162.2	163.8
Batch 1 (TCX)	0.1	104.3	139.7	129.5
Batch 2	0.1	72.1	123.2	144.9
Batch 3	0.1	75.5	130.9	128.4
Batch 4	0.1	102.3	131.2	135.1
Batch 5	0.1	79.3	115.0	129.2
Batch 6	0.1	57.7	130.9	113.3
Batch 7	0.1	65.1	110.6	120.2
Batch (Lab.) 5/83	0.1	81.1	138.3	169.7
Man. Batch 1 B	0.1	125.6	174.8	114.8
Man. Batch 2 B	0.1	103.5	175.3	118.6
Man. Batch 3 B	0.1	138.9	156.4	147.0
Man. Batch 3 B(Hydrate)	0.1	103.3	141.3	158.9
Glucomannan(Konjac plant)	0.1	103.3	69.9	108.6
Control SCM	0.1	100.0	100.0	100.0

These experiments were conducted to evaluate the cell response to different samples of Carrisyn® prepared under various conditions.

EXAMPLE 26

Acetyl Group Analysis:

The amount of O-acetyl groups in a series of different Carrisyn® batches was determined. Prior to analysis, the samples had been dried overnight in a vacuum oven at 40°C over P_2O_5. Solutions were then made of approximately 1 mg/ml in deionized water. The analytical procedure used was the same as that described by Hestrin (Shlomo Hestrin, J. Biol. Chem. (1949), 180, 249-261) involving the colorimetric observation of the ferric-acethydroxamic acid complex.

The results are as follows:

Table 13

Sample	mMoles acetyl groups Per gram Carrisyn®
1	3.72
3	1.30
4	4.15
3B	2.11
4B	0.23
5B	3.33
6B	2.28
8B	2.32
10B	1.68
531004	2.97
613006	2.77
701008	2.61
peracetylated(531004)	5.69
8B >100K	3.93
8B 10K <100K	2.98
3B 3K < 10K	0.10
3B < 3K	ND
methylated 8B	ND

A table of relative activity values (fibroblast stimulation) and acetylation for eight 0.1% Carrisyn® solutions at 72 hours measured on the same day follows below.

Table 14

Batch #	mMoles O-acetyl groups Per gram Carrisyn®	% Activity
613006	2.7700	136.0000
701008	2.6100	95.5000
1	3.7200	113.2000
3	1.3000	93.9000
4	4.1500	104.7000
3B	2.1100	111.7000
4B	0.2300	74.4000
5B	3.3300	100.7000

The data suggest a trend that Carrisyn® activity is directly proportional to the amount of O-acetyl groups per gram of Carrisyn®. This was a surprising and unexpected result.

A table of viscosity value (in centistokes) and acetylation for nine Carrisyn® samples follows below:

Table 15

Batch #	mMoles acetyl groups Per gram Carrisyn®	Viscosity (CentiStokes)
#1	3.7200	6.1000
#3	1.3000	1.1800
#4	4.1500	5.0900
#613006	2.7700	2.1200
#701008	2.6100	2.0800
#3B	2.1100	2.0200
#5B	3.3300	4.5300
#6B	2.2800	1.7500
#8B	2.3200	1.9700

This data shows that viscosity is exponentially proportional to the degree of acetylation of Carrisyn®. Viscosity can be used as a quality control measure for Carrisyn®. From the above data the following line of best fit was determined:

$$\text{Viscosity} = 0.496 \times e^{(0.604 \times \text{mMoles acetyl groups/g.})}$$

EXAMPLE 27

The following experiments were conducted with aloe leaves from Hill Top Gardens, Harlingen, Texas. The leaves were processed by washing, cutting, filleting, grinding, homogenization (1500 psi) and filtration (pulp filter media). The entire processing was completed in less than one hour and 20 minutes.

Part I

At 9:10 a.m., 3 liters of gel and 12 liters of 190 proof undenatured ethanol were mixed vigorously in a 5 gallon polypropylene bottle. This process was carried out in duplicate for a total of 6 liters of gel and 24 liters of ethanol. Five liters of the mixture were quickly transferred into each of the 5 stainless steel pans labelled 1 hr., 2 hrs., 4 hrs., 8 hrs., and 10 hrs. respectively. Another pan was included for 24 hrs. study.

After 1 hour from the time alcohol was added for example, the sample labelled 1 hour was concentrated by siphoning out the alcohol followed by centrifugation. The solid was washed in fresh alcohol which was decanted after centrifugation. The solid precipitate was placed in a lyophilizer bottle, quickly frozen in liquid nitrogen and set to lyophilize. The process of

removal of alcohol to the lyophilization step took an average of 50-60 minutes. Each labeled fraction was similarly treated.

Table 16 - For Part 1 Study

Sample # (hours)	Time	Time(put in lyophilizer)	Weight (gram)	(gram/ Liter)	Yield (W/V)
1	10:10a.m	11:15a.m.	.7176	.7176	.0718
2	11:10a.m.	12:10p.m.	.7643	.7643	.0764
4	1:10p.m.	2:15p.m.	.7748	.7748	.0775
8	5:10p.m.	6:10p.m.	.7903	.7903	.0790
10	7:10p.m.	7:40p.m.	.8147	.8147	.0815
24	9:10a.m.	10:05a.m.	.9373	.9373	.0937

Part II

Part II study was different from Part I by the fact that pure aloe gel was subjected to the time study. At the time X hours (X = 0, 1, 2, 4, 8 and 10 hours respectively). Two liters of pure gel were mixed with 8 liters of 190 proof undenatured alcohol. The mixture was agitated and allowed to settle for 4 hours before the alcohol would be removed by siphoning and centrifugation. The solid precipitate was treated as previously described in Part I.

Table 17 - For Part II Study

Sample #(hrs)	0	1	2	4	8	10
Time	9:10am	10:10am	11:10am	1:10pm	5:10pm	7:10pm
Time(to Remove Alcohol	1:10pm	2:10pm	3:10pm	5:10pm	9:10pm	over night
Time(hrs in Alco-hol	4	4	4	4	4	-
Time (into Lyophi-lizer	2:20pm	3:10pm	4:10pm	6:15pm	9:35pm	-
Dry Weight (gram)	1.4894	1.4752	1.4083	1.3443	1.4957	1.3324
Yield (gram/ liter) gel	.7447	.7376	.7042	.6722	.7478	.6662
Yield Percent (W/V)	.0745	.0738	.0704	.0672	.0748	.0666

TESTS ON TIME LAPSE STUDY CARRISYN®

Size Exclusion Chromatography (SEC):

The average molecular weight distribution of Carrisyn® was determined by size exclusion Chromatography (SEC). The liquid Chromatograph was a Hewlett Packard model 1084 B featuring an HP 79875 A UV-VIS detector, an autosampler 79841 A and a Biorad Model 1770 refractive index detector. The column was a Beckman prepacked column 7.5 X 300 mm packed with spherogel - TSK 2000 sw; part no. 244282, serial no. 5K526. The mobile phase was 0.05% (w/v) sodium azide solution with a flow rate of 0.5 ml/min at a temperature of 30°C. Dextran standards from Sigma Chemical Company, St. Louis, Missouri were used to establish the average molecular weight distribution as follows:

Dextran #	Lot #	Ave. M.W.	(mins.)
D-5376	24F-0298	2,000,000	11.21
D-1390	13F-0427	71,200	11.49
D-4133	114F-0335	40,600	11.84
D-9260	53F-0240	9,000	17.71

Applying the same conditions for the Carrisyn® separation, three major peaks were observed as follows:

HILLTOP® (freeze dried) FILLET:

Fraction #	Ret.(min)	% Total	% (3 Fractions)
1	10.87	10.20	12.2
2	16.38	17.73	21.1
3	22.48	56.02	66.7

HILLTOP® INSTANT PROCESS (Fast):

Fraction #	Ret.(min)	% Total	% (3 Fractions)
1	11.85	43.14	46.8
2	16.57	19.74	21.4
3	22.05	29.24	31.7

HILLTOP® Part I (1 hour):

Fraction #	Ret.(min)	% Total	% (3 Fractions)
1	11.52	50.30	54.6
2	16.28	14.04	15.2
3	21.99	27.71	30.1

HILLTOP® Part 2 (4 hours):

Fraction #	Ret.(min)	% Total	% (3 Fractions)
1	11.79	56.42	58.3
2	16.33	15.30	15.8
3	21.98	25.07	25.9

HILLTOP® Part 1 (10 hours):

Fraction #	Ret.(min)	% Total	% (3 Fractions)
1	11.14	57.73	60.4
2	16.37	14.66	15.5
3	22.03	22.44	23.7

HILLTOP® Part 1 (24 hours):

Fraction #	Ret.(min)	% Total	% (3 Fractions)
1	11.41	45.75	50.5
2	16.55	19.35	21.4
3	21.28	25.43	28.1

When Carrisyn® samples made earlier were separated under the same conditions as the above, three major fractions were also observed.

TCX MANUF. Batch #1

Fraction #	Ret.(min)	% Total	% (3 Fractions)
1	11.18	35.18	36.98
2	16.12	7.04	7.40
3	22.29	52.91	55.60

MEXICO Manuf. Batch #2

Fraction #	Ret.(min)	% Total	% (3 Fractions)
1	11.04	19.02	19.0
2	16.46	24.27	24.3
3	22.38	56.70	56.7

Based on the dextran standard, the average molecular weight distribution of the 3 fractions of Carrisyn® were as follows:

Fraction #1 $>$ 80,000
#2 $\geq$ 10,000
#3 $<$ 1,000

Only the average molecular weight distribution can be determined since dextrans and the polysaccharides of Carrisyn® may be physically and chemically different. For example, size separation may be influenced by other factors such as ionic charge, adsorption and/or partition between the solvents.

CALCIUM CONTENT OF CARRISYN®:

Although, the function of calcium in aloe activities is not completely established, this parameter is still monitored in Carrisyn®. The calcium content of Carrisyn® was determined by The Lancer Calcium Rapid Stat Kit which is generally used for the quantitative colorimetric determination of calcium in serum and urine. The deep blue calcium methylthymol blue complex formed is read at 612 nanometers. For this example, IBM UV/VIS spectrophotometer model 9420 was used to monitor the absorbance. Based on 3 point Calcium standard, the calcium content of Carrisyn® was determined as follows:

Part I

Sample # (hours in alcohol)	% Calcium (w/w)
0	N/A
1	4.58
2	5.03
4	5.06
8	5.06
10	5.39
24	5.40

Part II

Sample # (hours before alcohol)	% Calcium (w/w)
0	4.26
1	4.00
2	4.55
4	4.48
8	4.69
10	4.04

INFRARED ANALYSIS

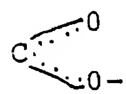
Infrared (IR) Spectroscopy as an analytical technique is especially important in the analysis of Carrisyn® whose viscosity in aqueous medium presents problems in any analysis requiring a solution.

Infrared analysis was carried out on Carrisyn® to monitor the functional groups. The instrument used was an IBM Fourier Transform - Infrared (FT-IR) spectrometer model 32. The

system status was, resolution=4; number of scans =32; detector=DTGS.

Since Carrisyn® is a powder it can be easily mixed with infrared grade potassium bromide (KBr) powder and the mixture pressed into a disc. The disc is then scanned. However, a new technique of making transparent films of 0.2% (w/v) aqueous Carrisyn® has been developed. The film gives a better infrared spectrum of Carrisyn® than the KBr disc technique. When Carrisyn™ was scanned from 4000 cm^{-1} to 400 cm^{-1}; the following characteristic absorption frequencies were observed:

Table 18

Peak #	Wave # (cm^{-1})	Functional Group	Vibrational Mode
1	1100-1035	O-H, C-O-C	δ (O-H)
2	3430-3390	O-H	v (O-H)
3	2930-2800	C-H	v (C-H)
4	1470-1380	C-H	δ (C-H)
5	1600-1550	COO^-, 	v (C=O)assym.
6	1450-1400		v (C=O)sym
7	1420-1410	C-O	
8	1650-1645	CONH	Amide I C=O
9	1550-1540	CONH	Amide II δ(N-H)
10	1746-1735	COOR	v (C-O)
11	1250-1240	COOR	v (C-O-C)
12	980-950	C-O O-CH_2	

With the above characteristic infrared frequencies, the absorption maxima of the frequencies in Part I and II studies were as follows:

Table 19

Part I and II IR Peak Maximum Wave #S (CM^{-1}) Arranged According to Intensity

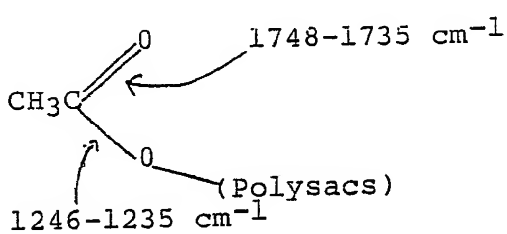
	1 hour	4 hours	8 hours	10 hours	24 hours
1.	1074.5	1068.7	1068.7	1587.6	1068.7
2.	1039.8	1037.8	1037.8	3370.1	1037.8
3.	3393.2	3412.5	3412.5	1076.4	3408.6

4.	1589.5	1242.3	1244.2	1037.8	1242.3
5.	1425.6	1740.0	1738.1	1421.7	1591.5
6.	1244.2	1373.5	1375.4	1246.2	1375.4
7.	1738.1	1637.8	1635.8	2042.9	1421.7
8.	603.8	1423.6	1425.6	1738.1	1738.1
9.	2926.4	603.8	601.9	603.8	669.4
10.	2039.0	2924.4	2924.4	2928.3	2924.4
11.	960.7	960.7	958.7	960.7	603.8

Part II

					0 Hours*
1.	1074.5	3397.1	1072.6	1068.7	1068.7
2.	3393.2	1072.6	1037.8	3408.6	1037.8
3.	1593.4	1593.4	3404.8	1244.2	3420.2
4.	1244.2	1560.6	1244.2	1734.2	1244.2
5.	1419.8	1244.2	1597.3	1635.8	1734.2
6.	1734.2	1419.8	1558.7	1653.2	1635.8
7.	2039.0	1736.2	1419.8	1375.4	1653.2
8.	638.5	2924.4	1375.4	2922.5	1375.4
9.	474.5	472.6	1740.0	472.6	472.6
10.	2927.4	607.7	605.7	1558.7	1419.8
11.	960.7	960.7	669.4	1419.8	1558.7
12.	873.9	875.8	2924.4	1541.3	605.7
				603.8	2924.4
					541.3

From an earlier infrared scan of Carrisyn®, deacetylated Carrisyn® and acetylated Carrisyn®, the carboxylate carbonyl bonds and the ester carbonyl frequencies were identified. The carbonyl functional group and the C-O-C stretches of the acetylated Carrisyn® were located between 1748-1735 cm^{-1} and 1246-1235 cm^{-1} respectively. The carboxylate carbonyl stretches of the deacetylated Carrisyn® were observed between 1600-1550 cm^{-1} and 1450-1400 cm^{-1} respectively.



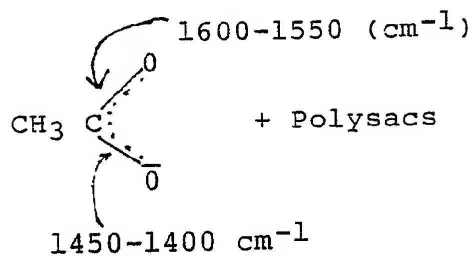


Table 20

Carrisyn®531	Carrisyn®531 (deacetylated)	Carrisyn®531 (acetylated)
1591.5	1431.4	1244.2
3414.4	3400.9	1741.9
1072.6	1032.0	1064.8
1246.2	1062.9	3460.7
1740.0	1091.8	1375.4
1431.4	875.8	1647.4
603.8	1147.8	1433.3
2930.2	1645.5	2932.2
806.3	2924.4	1541.3
958.7	814.1	956.8
879.6	615.4	603.8

When Carrisyn® was deacetylated, the absence of the ester C-O-C stretch frequency between 1248-1235 cm^{-1} was observed. It was noticed that the largest peak of the spectrum was centered at 1431 cm^{-1}. The degree of acetylation/deacetylation of Carrisyn® may be monitored by these two peaks. Actually, for this particular example, the peak at 1431 cm^{-1} (1560-1339 cm^{-1}) was contaminated by amide II peak since amide I could be observed at 1646 cm^{-1}.

However, Table 21 illustrates the absorption ratios of these two peaks for the Part I and Part II study.

Table 21

Sample # Hours	1450-1400 (cm^{-1}) Abs	12480-1240 (cm^{-1}) Abs	Abs. Ratio
	Part I		
1	.585	.584	.998
2	.607	.600	.988
4	.289	.487	1.685
8	.289	.450	1.557
10	.387	.326	0.842
24	.248	.308	1.242

Part II

0	.278	.475	1.709
1	.496	.513	1.034
2	N/A.	N/A	N/A
4	.452	.497	1.10
8	.437	.524	1.20
10	.291	.436	1.498

About 35 gallons of aloe vera gel were processed for the above characterization study. The process from washing through to filtration lasted approximately one hour and 20 minutes. This study demonstrated no noticeable degradation of the gel within this time period.

The alcohol precipitation step was shown to be an important factor in the quality of Carrisyn® produced. While Carrisyn® yield was increased by longer settling time in alcohol, the quality of the Carrisyn® produced may not be ideal for cell proliferation. It is believed that a time of 4-5 hours in alcohol is optimium for a good yield without adverse degradation.

It was observed in the Part II study that Carrisyn® yield is highest if alcohol precipitation is carried out immediately. Very little adverse effect on the cells or change in infrared spectrum was noticed if alcohol was added within 4 hours.

Infrared spectroscopy may offer the best technique to monitor the quality of Carrisyn® produced. The infrared spectra give characteristic strong ester carbonyl absorption peaks while the deacetylated Carrisyn® resulted in no such peaks. It is believed that the degree of deacetylation may be monitored by this technique. The only problem with this technique is that the presence of amides may influence the value of the carboxylate absorption peaks.

EXAMPLE 28

Figures 8-13 show infrared spectra for six different samples of Carrisyn® obtained from the Aloe barbadensis Miller plant by the process of the invention. As may easily be seen, the spectrum for each sample shows between 5 and 7 well resolved peaks from a wavenumber of about 1750 to a wavenumber of about

1250. As discussed above in Example 24, these peaks are indicative of the following functional groups: RCOO-, R-$NHCOCH_3$ and R-$OOCCH_3$. It is these functional groups which are believed to give Carrisyn® its activity. Thus, Figures 8-13 demonstrate that a consistent Carrisyn® product is produced from the leaves of the Aloe barbadensis Miller plant by the process of the present invention.

EXAMPLE 29

Figure 14 shows an infrared spectrum for raw aloe vera gel that was cast into a film. This spectrum also shows between 5 and 7 well resolved peaks from a wavenumber of about 1750 to a wavenumber of about 1250. Thus, Figure 14 provides evidence that Carrisyn® is present in the raw gel from the Aloe vera plant.

EXAMPLE 30

Figure 15 shows an infrared spectrum for a sample of Carrisyn®. As will be noted, the characteristic well-resolved peaks between a wavenumber of 1750 and a wavenumber of 1250 are almost absent. It is believed that this sample of Carrisyn® was precipitated from Aloe vera gel or juice with reused alcohol which somehow reduced the precipitation of Carrisyn®.

EXAMPLE 31

Figures 16-17 demonstrate the importance of extracting Carrisyn® from Aloe juice with alcohol as quickly as is practicable after preparation of the Aloe juice. Figure 16 is a spectrum of a Carrisyn® sample that was extracted with alcohol from Aloe juice one hour after the Aloe juice was prepared. Figure 17 is a spectrum of a Carrisyn® sample that was extracted with alcohol from Aloe juice ten hours after the Aloe juice was prepared. The characteristic well-resolved peaks between a wavenumber of 1750 and a wavenumber of 1250 show a significantly higher absorbance level in the spectrum of Figure 16 compared to the spectrum of Figure 17. Thus, better quality Carrisyn® appears to be produced the sooner Carrisyn® is extracted with alcohol from Aloe juice.

EXAMPLE 32

Figures 18-20 were constructed from the data listed in Table 22 below.

Table 22-Time Involved for Various Processes during the Production of Several Manufactured Carrisyn® Batches

Batch	Date Of Manufacture	Leaf Processing Time(min)	Homogenization Time(min)	Filtration Time (min)	Alcohol Addition Time (min)
1	8/6/85	75	8	152	15
2	8/23/85	270	15	90	6
3	9/18/85	404	21	225	60
4	9/20/85	242	29	240	60
5	9/23/85	209	27	245	60
6	9/26/85	375	30	255	60
7	10/1/85	200	15	66	60
1B	11/25/85	72	15	14	--
2B	12/3/85	41	20	12	--
3B	12/17/85	73	18	149	--
4B	1/7/86	137	28	364	15
5B	1/10/86	146	30	63	10
6B	1/24/86	113	25	105	--
7B	2/5/86	91	25	25	13
8B	2/18/86	143	26	44	13
9B	2/26/86	103	32	29	15
10B	3/18/86	119	75	104	15

Batch	Precipitation Time (min)	$1/T_{ppt} \times 10^4$ (min)	Total Time[1] Before Precipitation	$1/T_{total} \times 10^3$ (min)
1	1210	8.26	255	3.92
2	1095	9.13	384	2.60
3	790	12.7	700	1.43

4	790	12.7	535	1.87
5	870	11.5	526	1.90
6	660	15.2	695	1.44
7	1035	9.66	345	2.90
1B	283	35.3	117	8.54
2B	375	26.7	76	13.16
3B	270	37.0	252	3.97
4B	960	10.4	542	1.85
5B	250	40.0	255	3.92
6B	250	40.0	253	3.95
7B	285	35.0	144	6.94
8B	227	44.1	230	4.35
9B	240	41.7	167	5.99
10B	255	39.2	185	5.41

Batch	Total Time[2] For Homogenization & Filtration	$1/T_{gel} \times 10^3$ (min)	Yield (GRAMS)	% Yield (w/v)
1	160	6.3	36.7	0.054%
2	105	9.5	80.0	0.106%
3	246	4.1	71.1	0.075%
4	269	3.7	22.0	0.023%
5	272	3.7	17.8	0.018%
6	285	3.5	15.3	0.016%
7	81	12.3	88.7	0.098%
1B	29	34.5	34.3	0.065%
2B	32	31.3	13.8	0.041%
3B	167	6.0	177.3	0.117%
4B	392	2.6	81.8	0.054%
5B	93	10.8	145.0	0.096%
6B	130	7.7	120.3	0.074%
7B	50	20.0	184.9	0.122%
8B	70	14.3	109.8	0.073%
9B	61	16.4	188.7	0.125%
10B	179	5.6	151.5	0.100%

1 Total time from the cut on the first Aloe leaf until

the first drop of alcohol is added to the resulting gel.
[2] The total time for homogenization and filtration of the gel only.

As shown in Figure 18, Carrisyn® yield does not appear to be related to the time the gel is in alcohol. As shown in Figure 19, Carrisyn® yield is inversely proportional to the total process time before precipitation. As shown in Figure 20, Carrisyn® yield is inversely proportional to the time of homogenization and filtration. Thus, for highest Carrisyn® yields, the total process time before precipitation, more specifically the the time of homogenization and filtration, should be minimized to the extent possible.

EXAMPLE 33

Figure 21 shows an infrared spectrum for the alcohol precipitation product of the juice from *Aloe ferox*. This spectrum shows several well resolved peaks from a wavenumber of about 1750 to a wavenumber of about 1250. Thus, the alcohol precipitation product of the juice from *Aloe ferox* may have similar activity as Carrisyn®.

EXAMPLE 34

Figure 22 shows an infrared spectrum for the alcohol precipitation product of the juice from *Aloe africana*. This spectrum shows several well resolved peaks from a wavenumber of 1750 to a wavenumber of 1250. Thus, the alcohol precipitation product of the juice from *Aloe africana* may have similar activity as Carrisyn®.

EXAMPLE 35

Figure 23 shows an infrared spectrum for the alcohol precipitation product of the juice from *Africana ferox*, a hybrid of *Aloe ferox* and *Aloe africana*. This spectrum shows several well resolved peaks from a wavenumber of 1750 to a wavenumber of 1250. Thus, the alcohol precipitation product of the juice from *Africana ferox* may have similar activity as Carrisyn®.

EXAMPLE 36

Figure 24 shows an infrared spectrum for the alcohol precipitation product of the juice from Aloe dichotoma. This spectrum shows some peaks from a wavenumber of 1750 to a wavenumber of 1250. The alcohol precipitation product of the juice from Aloe dichotoma may have some similar activity as Carrisyn®.

EXAMPLE 37

Figures 25-29 show infrared spectra for cellulose, polygalacturonic acid, glucomannan from the Konjac plant, dextran and guar gum, respectively. These spectra show some peaks from a wavenumber of 1750 to a wavenumber of 1250 but not the sharp well-resolved peaks of Carrisyn® as in Figure 8. Thus, it is not believed that cellulose, polygalacturonic acid, glucomannan from the Konjac plant, dextran or guar gum would have the same activity as Carrisyn®.

EXAMPLE 38

Modification of rheological characteristics of Carrisyn®:

The modifications of Carrisyn® discussed below may affect one or more of the following rheological characteristics of Carrisyn®: absorption, penetration, specificity, solubility, viscosity, stablility, activity, or the target tissue. These modifications are important in drug targeting. Infrared spectra of the following modified forms of Carrisyn® have been produced. It is inferred that modified forms of Carrisyn® may possess similar biological activity as unmodified Carrisyn®.

A. Hydrogen Peroxide Treatment of Carrisyn®

1.00 g. of Carrisyn® (light pink-brown color, Lot #8B) was placed in a 1 L round bottom flask containing 500 ml of deionized water and a 1" stir bar. The mixture was stirred at ambient temperature for 30 minutes, and then in a cold room (4°C) for 30 minutes to give a slightly viscous, heterogeneous mixture containing some undissolved Carrisyn®. To this vigorously stirred mixture was added dropwise at 4°C, 5 ml of 30% H_2O_2

solution (Mallinckrodt, AR grade, #5240). There were no observed changes or gas evolution. The mixture was stirred gently at 4°C for 16 hours. No physical change was observed.

Solid sodium bisulfite (granular, Baker, #1-3556) (4.588 g., 1.0 equivalent with respect to H_2O_2) was dissolved in 30 ml H_2O. The resultant clear solution was added dropwise in 20 minutes to the stirring reaction mixture. During the addition, the reaction pH was monitored by pH paper and kept at pH 7 ± 0.5 using sodium bicarbonate solution (1 M, Fisher, #S-233). After complete addition, the mixture was stirred for 1 hour at ambient temperature, then concentrated on a rotary evaporator under reduced pressure at 35-40°C to a volume of approximately 80 ml. This concentrate was placed in pre-washed dialysis tubing (approximately 30 cm long, dry cylinder diameter 28.6 mm, Spectrapor Medical Indust., Inc. Los Angeles, CA #132633, 2000 MW cutoff) and dialyzed against deionized water in a 6 L Erlenmeyer flask at 4°C for 24 hours with water changes every six hours. The tubing was opened and the contents freeze-dried under vacuum for 12 hours to give 420 mg. of an off-white powder.

B. Sodium Periodate Cleavage* of Carrisyn®

1.00 g. of Carrisyn® (light pink-brown color, Lot #8B) was placed in a 1 L round bottom flask containing 500 ml of deionized water and a 1" stir bar. The mixture was stirred at ambient temperature for 30 minutes, then in a cold room (4°C) for 30 minutes to give a slightly viscous, heterogeneous mixture with some undissolved Carrisyn®. To this was added a solution of sodium periodate (99% Aldrich Chem. Co., #21,004-8; 59 mg, 5 mol % based on an average MW of 180/monomer) in 10 ml deionized water with vigorous stirring over 15 minutes. The mixture was stirred at 4°C for 15 hours. The solution became homogeneous and developed a light pink color. The pH was monitored with pH paper and kept at pH 7 ± 0.5.

Fifty (50) ml of ethylene glycol was added (three equivalents with respect to $NaIO_4$) and was stirred at room temperature for three hours to destroy unreacted $NaIO_4$. Solid $NaBH_4$

(630 mg, Aldrich Chem. Co., #19, 807-2, 98+%) was added in small portions with vigorous stirring over 30 minutes. Upon complete addition, the mixture was stirred at ambient temperature for 3 hours. Excess $NaBH_4$ was quenched with 20 ml acetone (distilled, 99.9+% HPLC grade, Aldrich Chemical Co., #27,072-5) at room temperature for 2 hours. The pH was >8, so it was adjusted to pH 7 with 50% aqueous HOAc solution (Fisher Chemical Co., HOAc reagent grade). The acetone was evaporated and the solution was concentrated on a rotary evaporator under reduced pressure at 35°-40°C to approximately 80 ml. The residue was transferred to dialysis tubing (approximately 30 cm long, dry cylinder diameter 28.6 mm, Spectrapor Medical Indust. Inc., Los Angeles, CA #132633, 2000 MW cutoff) that had been pre-washed with deionized water. The residue was dialyzed against deionized water in a 6 L Erlenmeyer flask for 24 hours with water changes every six hours.

The tubing contents was removed and freeze dried for twelve hours under vacuum to give 470 mg of a light brown powder.

*Experimental procedure taken in part from: J.M. Boffitt "Periodate Oxidation of Carbohydrate" in Adv. Carbohydrate Chemistry 11:1-41(1956).

C. Phosphorylation* of Carrisyn®

To a solution of sodium dihydrogen phosphate monohydrate (Matheson Coleman and Bell, CB742; 577 mg) and disodium hydrogen phosphate heptahydrate (Fisher Sci., S-373, Lot #855049; 837 mg) in 30 ml of deionized water was added 1.00 g of Carrisyn® (light pink-brown colored powder, Lot #8B). The heterogeneous mixture was stirred at 35°C for 30 minutes to give a pink-light brown paste. The water was evaporated on a rotary evaporator under reduced pressure at approximately 40°C. The residue was further dried at 0.1 mm Hg pressure in an oil pump for 3 hours. The hard solid was powdered with a glass rod and placed in a 1 L round bottom flask and then placed in a preheated oil bath at 155°C. A slow stream of argon (prepurified) was passed over the sample

which was stirred with a mechanical stirrer and a Teflon paddle. After three hours, the mixture was cooled to ambient temperature, slurried in 50 ml deionized H_2O and placed in dialysis tubing (30 cm long, dry cylinder diameter 28.6 mm, Spectrapor Medical Indust. Inc., Los Angeles, CA #132633, 2000 MW cutoff) which was prewashed with deionized H_2O. The mixture was dialyzed against deionized H_2O in a 6 L Erlenmeyer flask for 24 hours with H_2O changes every six hours in a cold room (4°C).

The tubing contents at approximately pH 7 were removed and freeze dried for 12 hours under vacuum to give 865 mg of a brown solid.

*Experimental procedure taken in part from: E.F. Paschall "Phosphation with Inorganic Phosphate Salts" in Methods in Carbohydrate Chemistry Vol. IV, pp. 294-296, 1964; R.L. Whistler, Ed., Academic Press, New York. For alternative procedure see, GA Towle and R.L. Whistler, Methods Carbohydrate Chem. 6, 408(1972);

D. Partial Methylation* of Carrisyn®

1.00 g of Carrisyn® (light pink-brown color, Lot #8B) was powdered in a mortar and pestle, and then suspended in 75 ml acetone (Fisher Sci., A-18, Lot #856136, distilled). With vigorous stirring, a 4.4 ml 30% NaOH solution made by dissolving NaOH pellets (Mallinckrodt AR grade, 7708, Lot KVHS) in deionized water and 2.2 ml dimethylsulfate (Fisher) were added simultaneously. The reagents were added in 4 equal portions at 15 minute intervals. After complete addition, the mixture was stirred for 15 minutes then evaporated on a rotary evaporator at reduced pressure. The residue was slurried in 50 ml of deionized H_2O and transferred to dialysis tubing (30 cm long, dry cylinder diameter 28.6 mm, Spectrapor Medical Indust. Inc., Los Angeles, CA #132633, 2000 MW cutoff) which had been pre-washed with deionized water. The residue was dialyzed against 5% $NaHCO_3$ solution ($NaHCO_3$ powder, Fisher, S-233, Lot #722534) in a 6 L Erlenmeyer flask at 4°C for 24 hours with a solution change every 6 hours. The residue was then dialyzed against deionized H_2O at

4°C for 36 hours in a 6 L Erlenmeyer flask with a H_2O change every 6 hrs.

The tubing contents at approximately pH 7 were removed and freeze dried for 12 hours under vacuum to give 759 mg. of a light brown powder.

*Experimental procedure taken in part from: E.L. Hirst and E. Percival. "Methylation of Polysaccharides and Fractionation of the Methylated Products" in Methods in Carbohydrate Chemistry, Vol. V, pp. 287-296, 1965; R.L. Whistler, Ed., Academic Press, New York.

Also see, W.N. Haworth, J. Chem. Soc. 107: 8-16 (1915).

E. Carboxymethylation* of Carrisyn®

1.00 g of Carrisyn® (light pink-brown colored solid, Lot #8B) was powdered in a mortar and pestle, and then suspended in 15 ml of isopropyl alcohol (ACS reagent grade, Aldrich Chem. Co. #19,076-4). With vigorous stirring, 0.5 g NaOH (Mallinckrodt AR grade, 7708, Lot #7708 KVHS) in 1 ml deionized H_2O was added dropwise over 5 minutes and was followed immediately by 140 mg of bromoacetic acid (Eastman Kodak Co., #964) in 1 ml of deionized H_2O. This mixture was stirred at room temperature under an argon stream for 80 minutes. The organic solvent was removed under reduced pressure on a rotary evaporator, in a bath temperature of 30°C. The resultant brown paste was diluted with 30 ml of deionized H_2O and the mixture was neutralized to pH 7 using 20% aqueous acetic acid (prepared from Fisher glacial acetic acid, ACS reagent grade, A-38). The product was transferred to dialysis tubing (30 cm. long, dry cylinder diameter 28.6 mm, Spectrapor Medical Indust. Inc., Los Angeles, CA #132633, 2000 MW cutoff) which was pre-washed with deionized H_2O. The product was dialyzed against deionized H_2O in a 6 L Erlenmeyer flask for 24 hrs. with H_2O changes every 6 hrs. in a cold room (4°C).

The tubing contents were removed and freeze dried for 12 hours under vacuum to give 609 mg of a brown, brittle solid.

*Experimental procedures taken in part from: Hans Vink, Die Makromolekulare Chemie 122:271-274 (1969).

F. Sulfation of Carrisyn®

To a 0° suspension of sulfur trioxide-trimethylamine complex (Aldrich Chem. Co., 13,587-9, white powder) in 50 ml of dimethylforamide (Baker Analyzed reagent grade, 3-9221) was added all at once 1.00 g of Carrisyn® (light pink-brown colored solid, Lot #8B, powdered in mortar/pestle) with vigorous stirring. After approximately 5 min., the mixture became very thick and difficult to stir. The mixture was maintained at 0° with stirring for 24 hours, and diluted with 50 ml deionized H_2O and transferred to dialysis tubing (30 cm long, dry cylinder diameter 28.6 mm, Spectrapor Medical Indust. Inc., Los Angeles, CA #132633, 2000 MW cutoff) which had been pre-washed with deionized H_2O. The mixture was dialyzed against 10% aqueous $NaHC_3$ solution (prepared from solid $NaHCO_3$, Fisher Sci. Co., ACS certified, S-233) in a 6 L Erlenmeyer flask at 4°C for 24 hrs., then against deionized H_2O at 0-4°C for 48 hrs. with H_2O changes every 6 hrs.

The tubing contents were removed and freeze dried for 12 hrs. under vacuum to give 575 mg of a hard brown solid.

*Experimental procedure taken in part from: R.L. Whistler and W.W. Spencer in "Sulfation by Triethylamine Sulfur Trioxide Complex", Methods in Carbohydrate Chemistry, Vol. IV, pp. 297-298, 1964; R.L. Whistler, Ed., Academic Press, New York.

For alternative procedures see, R.L. Whistler, Methods Carbohydrate Chem. VI, pp. 426-429, 1972.

G. Carrisyn® Crosslinking with Epichlorohydrin

1.00 g of Carrisyn® (light pink-brown colored solid, Lot #8B) was powdered in a mortar and pestle, then suspended in 200 ml of deionized H_2O. With vigorous stirring, epichlorohydrin (0.40 gm, Aldrich Chem. Co., 99%, gold label, Cat. #24,069-9) was added as a neat liquid followed by a solution of NaOH (Mallinckrodt AR grade, 7708, Lot #7708 KVHS 0.5gm) in 2.5 ml of deionized/degassed H_2O. The mixture was heated at 60°C for 80

min, then cooled to ambient temperature. The pH was adjusted to 7 with 20% aqueous acetic acid (prepared from Fisher glacial acetic acid, ACS reagent grade, A-38). The reaction mixture was transferred to dialysis tubing (30 cm long, dry cylinder diameter 28.6 mm., Spectrapor Medical Indust. Inc., Los Angeles, CA #132633, 2000 MW cutoff) which had been pre-washed with deionized H_2O. The reaction mixture was dialyzed against deionized H_2O in a 6 L Erlenmeyer flask for 24 hours with H_2O changes every six hours in a cold room at 4°C.

The tubing contents were removed and freeze dried for 12 hours under vacuum to give 485 mg of a light brown solid.

H. Formalin Crosslinked Carrisyn®

1.00 g of Carrisyn® (light pink-brown solid, Lot #8B) was powdered in a mortar and pestle, then suspended in 200 ml of deionized H_2O. To this suspension was added 3 ml of 37% formalin solution (Baker Analyzed Reagent grade, Lot #33674, Manuf. #2106, 37% formaldehyde) followed by 0.5 g NaOH (Mallinckrodt AR grade, 7708, Lot #7708 KVHS) in 2.5 ml deionized H_2O. The mixture was stirred vigorously at 60°C for 80 minutes. The mixture was cooled to room temperature, the pH was adjusted to 7 with 20% aqueous acetic acid (prepared from Fisher glacial acetic acid, ACS reagent grade, A-38), and the reaction mixture was transferred to dialysis tubing (30 cm. long, dry cylinder diameter 28.6 mm, Spectrapor Medical Indust. Inc., Los Angeles, CA #132633, 2000 MW cutoff). The mixture was dialyzed at 4°C in a 6 L Erlenmeyer flask against deionized H_2O with H_2O changes every 6 hours.

The tubing contents were removed and freeze dried for 12 hours under vacuum to give 518 mg of a white/brown and brittle solid.

EXAMPLE 39

Comparison of Common Topical Agents for Wound Treatment: Cytotoxicity for Human Fibroblasts in Culture

Cultures of human fibroblasts were used to determine the cytotoxicity of several topical agents that are commonly used for wound cleansing. The objective was to compare a wound gel containing Carrisyn™ with several standard cleansing agents that have different modes of action. These standard cleansing agents are designed to reduce bacterial damage and tissue breakdown following breach of the epidermal barrier. Release of radiolabeled chromium and uptake of trypan blue dye were used to measure cytotoxicity. The cultured fibroblasts were not damaged by concentrations of Carrisyn® as high as 0.5%. In contrast, povidone-iodine (Betadine®), trypsin and balsam of Peru (Granulex®), chlorhexidine (Hibiclens®), or hydrogen peroxide released ^{51}Cr from labeled cells. Betadine®, Hibiclens® and Granulex® also enhanced staining with trypan blue, but treatment with Carrisyn® did not. Based upon these in vitro studies, Carrisyn® appears to be safe for topical application and wound treatment.

Cell cultures. Human skin fibroblasts were grown from explants of newborn foreskins and from samples of adult skin collected from the lower abdomen at Caesarean section. The tissue was cleaned of fat and subcutaneous connective tissue, minced into 2 mm^3 particles and placed in small (25 cm^2) culture flasks. Culture medium, consisting of Minimum Essential Medium (MEM) (Inland Laboratories) supplemented with 5% fetal calf serum (Hazelton), 200 mM glutamine and 1% antibiotics, was added, and the cultures were maintained at 37°C in an atmosphere of 5% CO_2.

A mixture of keratinocytes and fibroblasts grew from the edges of the tissue within a few days. The keratinocytes failed to divide, but within 16-21 days the fibroblasts proliferated to form monolayers of elongated cells with a characteristic swirled pattern. All cultures were passaged at least three times before use and were used for up to 10-15 passages.

Topical agents. Several products that are commonly used to treat wounds and decubiti were added directly to standardized

cultures of human fibroblasts in order to measure cytotoxicity in an in vitro system. Povidone-iodine solution (Betadine®), hydrogen peroxide, Granulex® and chlorhexidine (Hibiclens®) are commonly used cleansing agents with different mechanisms of action. These compounds (0.001-0.5%) were compared with Carrisyn® for cytotoxic effects on cultured fibroblasts.

Treatment of cells. Cells from confluent monolayers were detached with 0.25% trypsin following a brief exposure (5-10 min) to Pucks-EDTA solution. The suspended cells were centrifuged to a pellet, washed once with fresh medium, and resuspended in MEM supplemented with glutamine, antibiotics and 1% fetal calf serum. Cell number was determined in an electronic cell counter (Coulter Electronics, Hialeah, FL) and adjusted by dilution as required for individual experiments. The cells were labeled with ^{51}Cr (1 μ Ci/ml) and plated in 24 well multiwell plates at a density of 10^5 per well. The plates were returned to the incubator for 18 hours. At the start of each experiment, radioactive media were removed by suction, and each well was washed 4 times with fresh MEM plus 1% fetal calf serum. MEM alone or MEM containing various dilutions of the test products was added to replicate wells, and the plates were incubated for 1-30 minutes longer. At the end of the incubation period, the media were collected and reserved for measurement of released radioactivity. The cells in each well were lysed by addition of 0.5 ml of Triton X-100 (1%) with 0.1 M NaOH, and samples of the lysates were taken for measurement of radioactivity.

Measurement of cytotoxicity. Cytotoxicity was quantitated by release of radioactive chromium from labeled fibroblasts incubated with the various different chemical agents. The percent release was calculated by dividing the amount of radioactivity in the supernatant media by the total radioactivity in both media and cells.

An alternate assessment of cytotoxicity was provided by staining with trypan blue. The cells were incubated with each of the test agents for 15 minutes. Trypan blue (1%) was added to each well and incubation was continued for 5 minutes longer. The

samples were inspected by light microscopy and photographed through a Nikon® 35 mm camera attached to a Nikon® inverted phase microscope. Cytotoxicity was estimated by determining the percentage of cells stained with trypan blue in comparison with control (untreated) cells. The results of this determination are shown in Table 23 below.

Table 23

Cytotoxicity of Topical Preparations
As Determined by Trypan Blue Staining

Product	Conc.	Inc. Time	Percent Staining
Betadine	0.01%	15 min.	100
Hibiclens	0.01%	15 min.	100
Granulex	0.01%	15 min.	100
Carrisyn™	0.01%	15 min.	5
Media Alone	---	15 min.	1

Cultured cells were incubated with each of the agents or with media alone for 15 minutes, trypan blue (1%) was applied, and after 5 minutes the number of stained nuclei were counted and expressed as percent of the total cell nuclei within the visual field.

Time course of cell injury. Direct cell injury by the topical agents occurred rapidly and was reflected by increased release of ^{51}Cr from the cells. Cultured fibroblasts that were treated with medium alone or with 10% fetal calf serum released no more than 5% of the total chromium label during incubation for 5-60 minutes. In contrast, cells treated with 0.05% Betadine®, Granulex® or Hibiclens® released between 55 and 62% of the total label within 5 minutes. Incubation for 10 or 15 minutes slightly increased the amount released for all of these agents, but longer incubation (30 minutes) did not further increase release of radioactivity. Cells treated with Carrisyn® (CDWG) (0.05%) released no more than 5% of the total label during incubation for as long as 30 minutes. (Figure 30)

Influence of concentration on cell injury. The various agents were tested for cytotoxicity in concentrations ranging from 0.005 to 0.05%. As shown in Figure 31, Granulex® and Hibiclens® (0.01%) released approximately 25% and 70% of the total chromium label from fibroblasts. Release by the lowest concentrations of Betadine® (0.005 and 0.01%) was no more than that by medium alone, but cells exposed to 0.015% Betadine® released more than 70% of their total radioactivity. Carrisyn® in concentrations up to 0.5% released no more than medium alone.

Similar results were obtained when trypan blue staining was used to assess cell injury. As shown in the table, incubation for 15 minutes with 0.01% Betadine®, Hibiclens® or Granulex® killed 100% of the cells. Incubation with Carrisyn® at the same concentration killed only 5%. Hydrogen peroxide at a concentration of 0.01% badly damaged the cells as judged by changes in morphology, but trypan blue staining by this agent could not be measured because of its decolorizing effects.

Effects of combined agents. In some experiments Carrisyn® was added to fibroblast cultures before addition of other topical agents to determine if it had a protective effect. The cytotoxic effect was measured in media alone and in media that contained 10% fetal calf serum. Figure 32 shows that although Carrisyn® alone did not damage the cells, it did not alter the amount of ^{51}Cr released by either Hibiclens® or Betadine® during a 15 minute incubation period. Similarly, inclusion of fetal calf serum in the incubation medium did not alter the cytotoxic effects of these agents.

EXAMPLE 40

An 83 year old female patient, TB, developed an ulcer, 25 mm in diameter, on the lateral margin of the left foot. The ulcer had been represent for several months, and had failed to respond to several treatment regimens.

The wound was treated with the product of Example 3 and the product of Example 7 using a t.i.d. treatment schedule. The clean wound was soaked for 15 minutes with the product of Example

3. Excessive product was absorbed from the wound with a dry sterile 4 X 4 gauze.

The product of Example 7 was then applied in a quantity sufficient to cover the wound and to prevent wound dehydration between dressing changes.

The progression of wound healing was measured by interval photographs and planimetry of the wound defect. The progression of wound closure is shown in Table 24.

Table 24 - Progression of Wound Healing

Day	Wound Area (Sq. In.)	Percentage of Healing
1	1.24	0.00
28	0.51	58.87
77	0.29	76.61
83	0.12	90.32
97	0.00	100.00

The epidermal defect was substantially closed in 12 weeks with complete closure occuring in 14 weeks.

EXAMPLE 41

A 32 year old patient, was presented with a history of ulcerative colitis for "many years". During an active episode, she had been unresponsive to a daily regimen of 40 mg Prednisone, 3 grams Asulfidine®, 50 mg 6-mercaptopurine, and Flagyl®. She continued to have a painful abdomen and 4-8 bloody bowel movements per day. She was placed on hyperalimentation. Endoscopic findings revealed severe ascending colon ulcerations with mild hepatic to transverse ulcerations. The patient was placed on 50 mg of Carrisyn® q.i.d. in addition to her other medications and sent home. In one week, her symptoms were virtually gone. The abdomen was mildly tender and endoscopy revealed a healed and mildly congested mucosa. The patient was slowly taken off other medications and the clinical picture continued to improve. The patient is currently maintained on Carrisyn™ as the sole medication at this time. Physical exam and symptoms are recorded as totally normal.

Five additional cases with similar responses to ulcera-

tive colitis and Crohn's disease have been seen. One patient ran out of Carrisyn® capsules. In four weeks, mild symptoms began to recur (there was increased stool with mild abdominal discomfort), and she returned for a supply of medication. In three days she was back to total normal bowel symptomatology.

EXAMPLE 42

A number of AIDS patients have received prolonged high doses of Carrisyn® without toxicity or side-effects. A rise in T-4 and T-8 lymphocyte ratios and an increase in absolute T-4 counts was seen in these AIDS patients with a reduction and elimination of clinical symptoms, as well as a reduction in opportunistic infections. It is suggested that Carrisyn® had an anti-viral or immune modulation effect in patients.

A stimulation to the lymphocytes of these patients has been observed which suggests that Carrisyn® may be involved in immune modulation.

EXAMPLE 43

Tic douloureux, or neuralgia of the fifth cranial nerve, is characterized by attacks of severe, unbearable pain over one or more branches of the trigeminal nerve. The pain usually is transient, and attacks may be precipitated by touching some area of the face - the so-called trigger zone.

The cause and cure of this painful disease are unknown. Several attempts to treat the disorder have met with little or no success. Various treatments have included analgesics, phenytoin, peripheral avulsion of the involved nerve branch as it leaves the skull, and injection of 98 percent alcohol into the gasserian ganglion.

A more drastic treatment - sectioning the sensory root of the nerve proximal to the ganglion - leaves the patient permanently without sensation in the area supplied by the sectioned nerve. Another recent treatment attempt uses carbamazepine and phenoliophendylate injections. However, these injections can be complicated by painful numbness and serious side effects.

None of the previously cited treatments is desirable.

A 43 year old woman was diagnosed as having tic douloureux. The affected area included the first and third divisions of the trigeminal nerve on the right side.

The patient could trigger the pain by brushing or combing her hair on the right side. She had been treated unsucessfully with diazepam (Valium®), antihistamines, analgesics, propranolol hydrochloride (Inderal®), and phenobarbital. The patient said she had not had a pain-free day since the onset of the disease.

The proposed therapy involved drinking 1 to 2 oz. of the product of Example 2 daily for three months. After that period, the therapy would be evaluated.

The patient's pain diminished significantly within two weeks of initiating therapy. She said she felt well for a few weeks. However, she then went on a two week trip, during which she did not drink the product of Example 2. The symptoms and pain returned during the trip. After she resumed the medication, however, the pain disappeared within a few days. For the next few weeks, she again felt well.

After drinking the juice daily for more than six months without interruption, she reports that she can brush and comb her hair without triggering the pain. Her appearance has improved, and she says she feels better than ever before.

EXAMPLE 44

The Effect of Alcohol Concentration on Carrisyn® Yield

PROCEDURE:

Hilltop leaves (15.9 lbs.) were washed, filleted and ground in a Waring blender, then filtered through eight layers of cotton cloth. The gel was then transferred to four 11 quart, stainless steel pans, and cold USP grade ethyl alcohol was added to each in two, three, four and five to one ratios by volume. The amounts can be summarized as follows:

Ratio (ethanol:aloe gel)	Amt. of Gel	Amt. of Ethyl Alcohol
2:1	500 ml	1000 ml
3:1	500 ml	1500 ml
4:1	1670 ml	6680 ml
5:1	500 ml	2500 ml

The precipitates were allowed to settle out for four hours, then the remaining alcohol-gel solutions were carefully decanted and saved in separate containers. The precipitates were centrifuged for 10 minutes at 2800 rpm using a IEC Centra-7 centrifuge, washed with alcohol, then centrifuged again under the same conditions. The pellets were transferred to 600 ml jars, frozen in liquid nitrogen, and lyophilized overnight.

An additional volume of alcohol was added to the supernatant from the 2:1 ratio and allowed to settle overnight at room temperature. The remaining supernatants were also left at room temperature and allowed to settle out overnight.

The following day, the precipitates were collected from the supernates as previously described with the exception of the pellet from the 2:1 ratio that had been precipitated with an additional volume of alcohol. In this case, approximately 5-10 ml of water was added as the pellet was transferred to the lyophilization jar.

Results:

The results of the initial, four hour alcohol precipitations can be summarized as follows:

Ratio (ethanol:aloe gel)	Yield(g)	%Yield (g. Carrisyn®/g. gel)
2:1	.0518	.010
3:1	.3847	.077
4:1	1.945	.116
5:1	.6675	.134

After addition of another volume of ethyl alcohol, the 2:1 supernate produced another 178 mg of Carrisyn®. Just by settling overnight, the 3:1 and 4:1 ratio supernates yielded another 89 and 105 mg, respectively. The 5:1 ratio yielded only

negligible precipitation after the initial isolation, and was thus not reharvested.

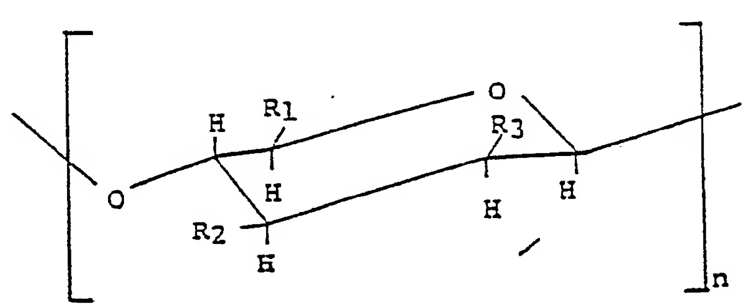
In the case of the second precipitation from the 2:1 ratio (3:1), 5-10 ml of water were used to rinse out the centrifuge bucket before lyophilization. This produced a white, fluffy Carrisyn® of low density that differed greatly from the denser, gray colored Carrisyn® samples that the other samples produced.

SUMMARY

Carrisyn® is predominantly a mannan as evidenced by Example 13 which shows that Carrisyn® consists of about 80% mannose. The mannose monomers of the mannan are connected largely by a β (1 → 4) linkage as evidenced by Example 14. The results from elemental analysis, IR, NMR and acetyl group analysis show that there are O-acetyl, N-acetyl and carboxylate functional groups on Carrisyn®.

The time it takes to process Carrisyn® prior to precipitation has been shown to be important on the overall quality and yield of the Carrisyn®. The longer the gel is allowed to stand before Carrisyn® is precipitated, the longer the enzymes in the gel have to work on Carrisyn® and cleave the functional groups and glycosidic bonds (β (1 → 4)) thus reducing or eliminating its activity.

The following structure was deduced from all of the above experimental results and evidence:



wherein R_1 = $-CH_2OH$, $-COO^-$, or $- CH_2OOCH_3$;

R_2 = $-OH$, $-OOCCH_3$, or $-NHCOCH_3$;

R_3 = $-OH$, $-OOCCH_3$, or $-NHCOCH_3$; and

n = 2 to about 50,000.

The predominantly mannan chain may include other substituted simple pentose or hexose monomers.

The production, isolation and characterization of Carrisyn® involved novel and unobvious procedures and techniques. As evidenced by the prior work of others in this field, which prior work was incorrect or inaccurate for one reason or another, Carrisyn® is novel and unobvious from the prior attempts of others to isolate and characterize the active substance in Aloe.

CLAIMS

What is claimed is:

1. A process for extracting the active chemical substance in the aloe plant from a leaf of the aloe plant, comprising steps of:

(a) obtaining aloe juice having solubilized matter;

(b) adding a water soluble, lower aliphatic polar solvent to the aloe juice to precipitate the active chemical substance and thereby to form a heterogeneous solution;

(c) removing the water soluble, lower aliphatic polar solvent and the solubilized matter from the heterogeneous solution to isolate the precipitated active chemical substance; and

(d) drying the precipitated active chemical substance.

2. A process for extracting the active chemical substance in the aloe plant from a leaf of the aloe plant according to Claim 1, wherein said aloe juice is obtained by:

(a) washing an aloe leaf in a bactericidal solution to remove substantilly all surface dirt and bacteria;

(b) removing at least a first end portion from said washed leaf;

(c) draining, preserving and collecting anthraquinone rich sap from said cut and washed leaf;

(d) removing rind from said leaf to produce a substantially anthroquinone-free gel fillet; and

(e) grinding and homogenizing said substantially anthraquinone-free aloe gel fillet to produce substantially anthraquinone-free aloe juice having solubilized matter.

3. A process for extracting the active chemical substance in the aloe plant from a leaf of the aloe plant according to Claim 1, wherein said aloe juice is obtained by:

(a) washing an aloe leaf in a bactericidal solution to remove substantially all surface dirt and bacteria;

(b) crushing the washed aloe leaf;

(c) dialyzing the crushed leaf chemically to remove and separate a substantially anthraquinone-free aloe gel from remaining fractions of the crushed leaf; and

(d) grinding and homogenizing said substantially anthraquinone-free aloe gel to produce substantially anthraquinone-free aloe juice having solubilized matter.

4. A process for extracting the active chemical substance in the aloe plant from a leaf of the aloe plant according to Claim 1, wherein said aloe juice is obtained by:

(a) washing an aloe leaf in a bactericidal solution to remove substantially all surface dirt and bacteria;

(b) crushing the washed aloe leaf; and

(c) grinding and homogenizing said crushed aloe leaf to produce aloe juice having solubilized matter.

5. A process for extracting the active chemical substance in the aloe plant from a leaf of the aloe plant according to Claim 1, wherein said aloe juice is obtained by:

(a) washing an aloe leaf in a bactericidal solution to remove surface dirt and bacteria;

(b) grinding said aloe leaf;

(c) filtering said ground aloe leaf to remove fibrous material; and

(d) homogenizing said ground aloe leaf to produce an anthraquinone-rich aloe juice having solubilized matter.

6. A process for extracting the active chemical substance in the aloe plant from a leaf of the aloe plant according to Claim 1, wherein said aloe juice is obtained by:

(a) crushing a leaf of an aloe plant to extrude aloe juice having solubilized matter.

7. A process for extracting the active chemical substance in the aloe plant from a leaf of the aloe plant according to Claim 1, wherein said aloe juice is obtained by:

(a) washing an aloe leaf in a bactericidal solution to remove substantially all surface dirt and bacteria;

(b) removing rind from said leaf to produce an aloe gel fillet; and

(c) grinding and homogenizing said aloe gel fillet to produce aloe juice having solubilized matter.

8. A product produced by the process of Claim 1.

9. Composition of matter, comprising:

an ethanol precipitation product of juice of the aloe plant wherein said precipitation product is substantially non-degradable and lyophilized.

10. Composition of matter according to Claim 9, wherein said ethanol precipitation product is obtained from juice of the aloe plant in its variety Aloe vera.

11. Composition of matter according to Claim 9, wherein said ethanol precipitation product is obtained from juice of the aloe plant in its variety Aloe ferox.

12. Composition of matter according to Claim 9, wherein said ethanol precipitation product is obtained from juice of the aloe plant in its variety Aloe africana.

13. Composition of matter according to Claim 9, wherein said ethanol precipitation product is obtained from juice of the aloe plant in its variety Africana ferox.

14. Composition of matter according to Claim 9, wherein said ethanol precipitation product is obtained from juice of the aloe plant in its variety Aloe dichotoma.

15. A process for extracting the active chemical substance in the aloe plant from a leaf of the aloe plant according to Claim 1, wherein said aloe juice is obtained by:

(a) removing rind from said leaf to produce an aloe gel fillet;

(b) grinding and homogenizing said aloe gel fillet to produce aloe juice having solubilized matter; and

(c) filtering said aloe juice to remove fibrous material.

16. A process for extracting the active chemical substance in the aloe plant from a leaf of the aloe plant according to Claim 15, wherein immediately prior to said filtering step, said ground and homogenized aloe juice is dialyzed chemically to remove and separate a substantially anthraquinone-free aloe juice.

17. A base formulation for the production of Aloe vera-containing topical products, comprising:

(a) a thickener;

(b) a food preservative; and

(c) a substantially anthraquinone-free aloe juice.

18. The formulation of Claim 17 wherein the thickener is methylparaben and the food preservative is potassium sorbate.

19. The formulation of Claim 18 wherein the weight ratio of methylparaben to potassium sorbate to raw Aloe vera gel is about 1.79:1:1000.

20. A moisture-resistant ointment emulsion formulation for topical to skin having an aqueous component phase and an oil component phase, comprising:

(A) for the aqueous phase:

(1) a substantially anthraquinone-free aloe juice;

(2) an agent to reduce skin surface tension and to permit adsorption on the skin of said aloe juice;

(3) an active agent to promote healing of the skin;

(4) a thickener for the water phase;

(5) one or more preservatives for the water phase; and a

(6) pH adjuster for the aqueous phase; and

(B) for the oil phase:

(1) one or more oil barrier agents to prevent the removal of the aqueous phase from the skin by contact of the skin by an aqueous-containing solution;

(2) one or more thickeners for the oil phase;

(3) a mixing agent for the oil phase;
(4) a skin softener;
(5) an oil base for the oil phase; and
(6) a surfactant.

21. The moisture-resistant ointment of Claim 20, wherein the oil phase additionally comprises:
(a) a agent to promote skin cell metabolism;
(b) a protein for wound healing; and
(c) a fragrance.

22. The moisture-resistant ointment of Claim 20, wherein:

(A) in the aqueous phase:

(1) the agent to reduce skin surface tension and to permit adsorption on the skin of the aloe juice is propyleneglycol;

(2) the active agent to promote healing of the skin is allantoin;

(3) the thickener for the water phase is methylparaben;

(4) the preservatives for the aqueous phase are imidazolidinyl urea and sorbitol; and

(5) the pH adjuster is pH adjuster is phosphoric acid; and

(B) in the oil phase:

(1) the barrier agents are glyceryl stearate and stearyl stearate;

(2) the thickeners for the oil phase are dioctyl adipate, octyl stearate and octyl palmitate and propylparaben;

(3) the skin softener is lanolin;

(4) the oil phase is mineral oil; and

(5) the surfactant is dimethicone.

23 . The moisture-resistant ointment of Claim 21, wherein in the oil phase:

(a) the agent to promote skin cell metabolism is Vitamin E;

(b) the protein for wound healing is cocamidopropyldimonium hydroxypropylamino collagen.

24. The moisture-resistant ointment of Claim 22 wherein:

(a) in the aqueous phase, the weight ratio of propylene glycol to allantoin to methylparaben to imidazolidinyl urea to sorbitol to phosphoric acid is 17:1:0.75:0.002:1:1; and

(b) in the oil phase, the weight ratio of glyceryl stearate to stearyl stearate to dioctyl adipate and octyl stearate and octyl palmitate to palita midopropyl dimethylamine to cetyl alcohol to lanolin to mineral oil to dimethicone to propylparaben is about 36:9:9:3.5:3.5:3.5:3.5:1:0.3.

25. The moisture-resistant ointment of Claim 23, wherein the ratio of Vitamin E to cocamidopropyldimonium hydroxypropylamino collagen is 1:1.

26. An aloe-based counter-irritant formulation having a water phase and an oil phase for treatment of soft-tissue skin injury, comprising:

(A) A carrier slurry for the water phase, comprising a water-miscible thickener;

(B) A water phase, comprising:

(1) a substantially anthraquinone-free aloe juice;

(2) an agent to reduce surface tension and to permit adsorption on the skin of the aloe juice;

(3) a surfactant-emulsifier and pH adjuster;

(4) an active agent to promote healing of the skin;

(5) a thickener for the water phase; and

(6) one or more preservatives;

(C) An oil phase, comprising:

(1) an extended wear vehicle for an oil base;

(2) an oil base;

(3) one or more thickeners for the oil phase;

(4) a mixing agent;

(5) one or more active counter-irritant agents; and

(6) an anti-inflammatory agent.

27. The counter-irritant formulation of Claim 26, wherein

(A) The water miscible thickener is a water-soluble resin used to form gels consisting of cross-linked, high molecular weight acrylic acid polymers;

(B) In the water phase,

(1) the agent to reduce surface tension and to permit adsorption on the skin of the aloe juice is propyleneglycol;

(2) the surfactant-emulsifier and pH adjuster is triethanolamine;

(3) the active agent to promote healing of the skin is allantoin;

(4) the thickener for the water pahse is methylparaben; and

(5) the preservatives comprise potassium sorbate and urea; and

(C) In the oil phase,

(1) the extended wear vehicle for the oil base is petroleum USP (white);

(2) the oil base comprises Carnation (white) mineral oil, mineral oil, lanolin alcohol, and acetylated lanolin alcohol;

(3) the thickeners for the oil phase comprise stearic acid, glyceryl stearate, dioctyl adipate, octyl stearate, octyl palmitate, white oleic acid, stearyl stearate, propylparaben, and butylparaben;

(4) the mixing agent is cetyl alcohol;

(5) the counter-irritant agents are eucalyptus oil, methyl salicylate, camphor, and methol; and

(6) the anti-inflammatory agent is histamine dihydrochloride.

28. The counter-irritant formulation of Claim 27, wherein the ratio of carbohydrate polymer to propylene glycol to triethanolamine to allantoin to methylparaben to potassium sorbate to urea to petroleum USP (white) to Carnation (white) mineral oil to mineral oil and lanolin alcohol to acetylated lanolin alcohol to stearic acid to glyceryl stearate to dioctyl adipate and octyl stearate and octyl palmitate to white oleic acid to stearyl stearate to propyl paraben to butylparaben to cetyl alcohol to eucalyptus oil to methylsalicylate to camphor to methol to histamine dihydrochloride is about 4.4:157:55:10:1: 0.55:22:8:8:7.5:7.5:23.5:10.3:10.3:1:1:1:1:8.2:22:136:22:22.5.

29. A. composition of matter, comprising:

(a) 0.1% to 100% wt. % substantially-free aloe juice;

(b) less than 2 wt.% allantoin;

(c) less than 6 wt.% panthenol;

(d) a water-miscible thickener as a carrier;

(e) a nutrient for cell growth; and

(f) one or more surfactant-emulsifiers and pH adjusters; and

(g) one or more preservatives.

30. The composition of Claim 29 comprising:

(a) a water-soluble resin used to form gels consisting of cross-linked, high-molecular weight acrylic acid polymers as the water-miscible thickener and carrier;

(b) a substantially anthraquinone-free aloe juice;

(c) panthenol;

(d) allantoin;

(e) L-glutamic acid as the nutrient for cell growth;

(f) imidazolidinyl urea, potassium sorbate and sodium benzoate as preservatives;

(g) triethanolamine as a surfactant emulsifier and alkalizing agent; and

(h) L-glutamic acid as a sweetener.

31. The composition of Claim 30 wherein the weight ratio of

(a) the hydrophilic aliphatic polymer;
(b) the substantially anthraquinone-free aloe juice;
(c) the panthenol;
(d) the allantoin;
(e) the L-glutamic acid;
(f) the imidazolidinyl urea;
(g) the potassium sorbate;
(h) the sodium benzoate; and
(i) the triethanolamine

is approximately 0.7:95.45:20.0:0.6:0.3:0.1:0.05:0.1:0.7.

32. A composition of matter for drinking comprising:

(a) 0.1 to 100 wt % substantially anthraquinone-free aloe juice;
(b) sodium benzoate and potassium sorbate as food preservatives;
(c) citric acid;
(d) Vitamin E;
(e) glycine as a sweetener; and
(f) vanilla and cinnamon oil as flavorants.

33. The composition of Claim 32, wherein the weight ratio of

(a) the substantially anthraquinone free aloe juice,
(b) the sodium benzoate,
(c) the potassium sorbate,
(d) the citric acid,
(e) the Vitamin E,
(f) the glycine,
(g) the vanilla, and
(h) the cinnamon oil

is approximately 989.011:0.986:0.493:1.085:0.261:7.829:0.316:0.019.

34. A process for producing substantially anthraquinone-free aloe gel from a leaf of the aloe plant comprising:

a) washing an aloe leaf in a bactericidal solution to remove substantially all surface dirt and bacteria;

b) crushing the washed aloe leaf; and

c) dialyzing the crushed leaf chemically to remove and separate a substantially anthraquinone-free gel from remaining fractions of the crushed leaf.

35. A process for producing substantially anthraquinone - free aloe gel from a leaf of the aloe plant, comprising:

a) crushing a leaf of an aloe plant; and

b) dialyzing the crushed leaf chemically to remove and separate a substantially anthraquinone-free aloe gel from remaining fractions of the crushed leaf.

36. A process for producing substantially anthraquinone - free juice from a leaf of the aloe plant comprising:

a) washing an aloe leaf in a bactericidal solution substantially to remove surface dirt and bacteria;

b) removing a first end portion and a second end portion from said washed leaf;

c) draining, preserving and collecting anthraquinone rich sap from said washed and cut leaf;

d) removing rind from said leaf to produce a substantially anthraquinone - free aloe gel fillet; and

e) grinding and homogenizing said substantially anthraquinone - free aloe gel fillet to produce substantially anthraquinone - free aloe juice.

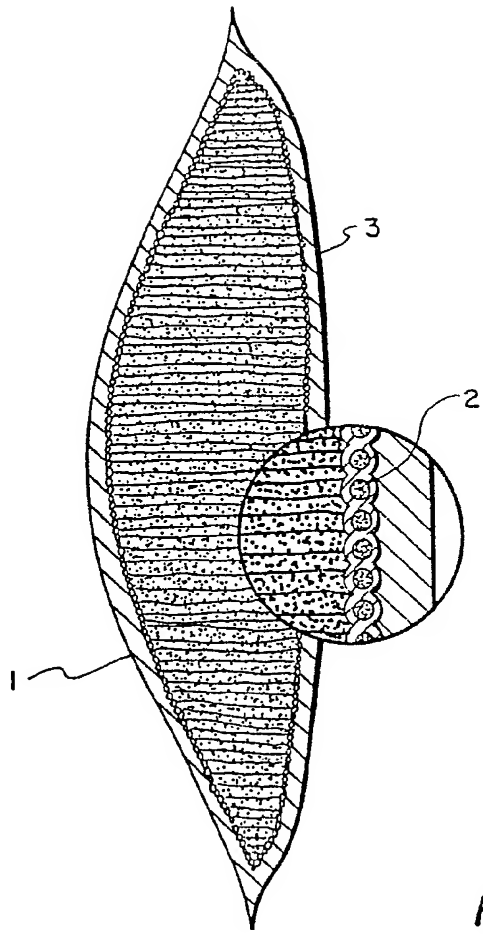


FIG. 1

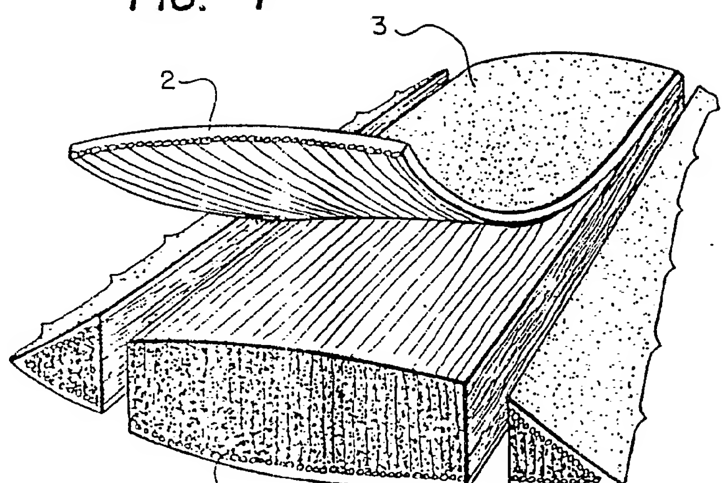


FIG. 2

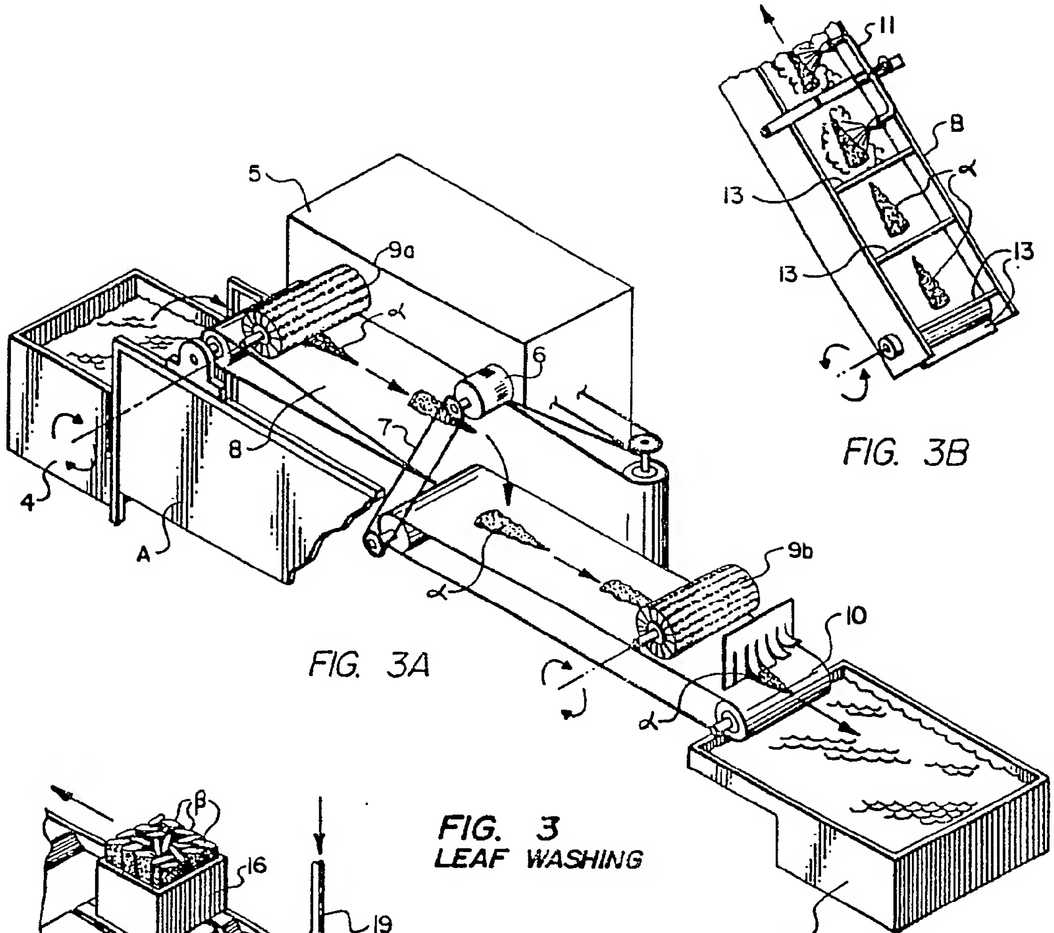


FIG. 3A

FIG. 3B

FIG. 3
LEAF WASHING

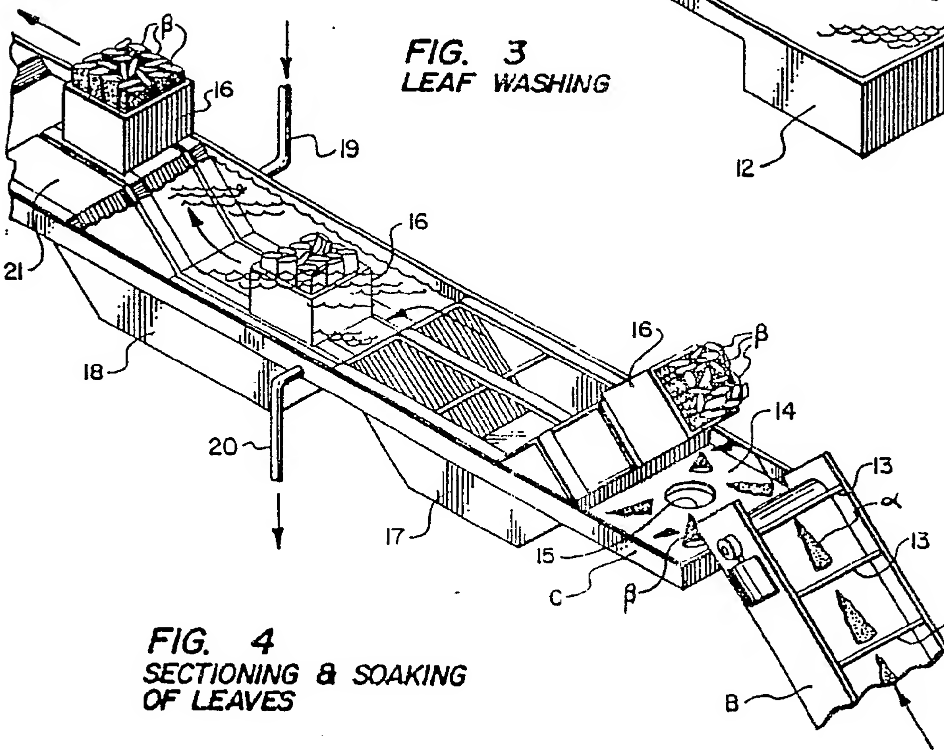


FIG. 4
SECTIONING & SOAKING
OF LEAVES

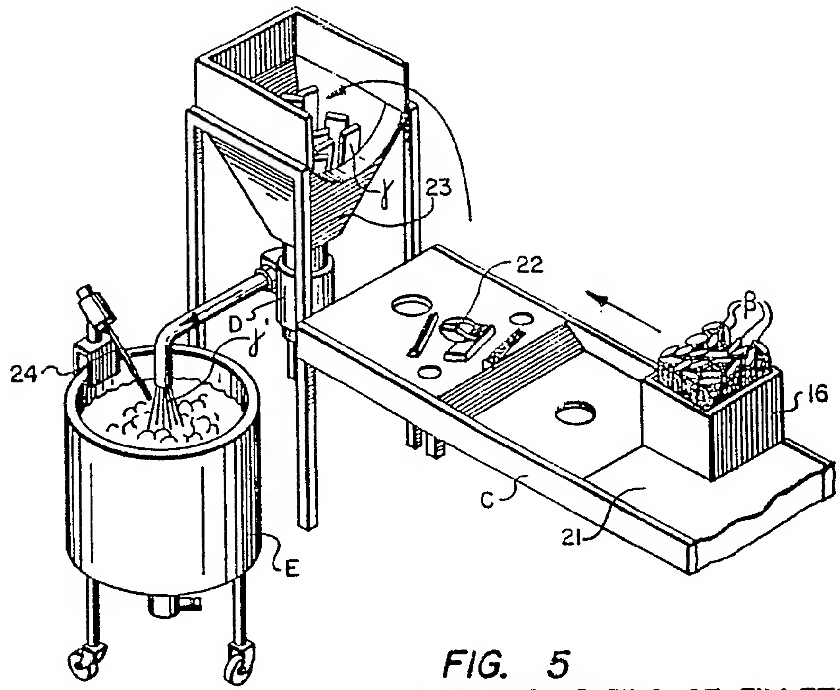
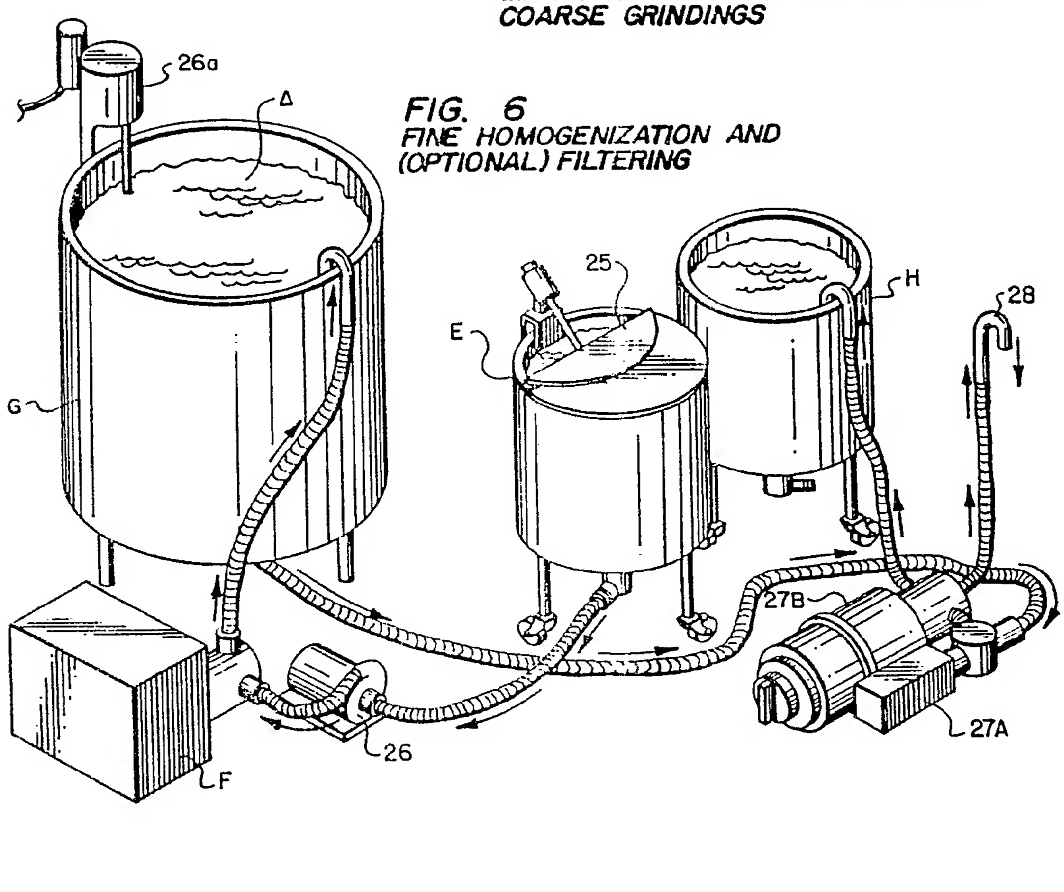


FIG. 5
MANUFACTURING OF FILLETS AND COARSE GRINDINGS

FIG. 6
FINE HOMOGENIZATION AND (OPTIONAL) FILTERING



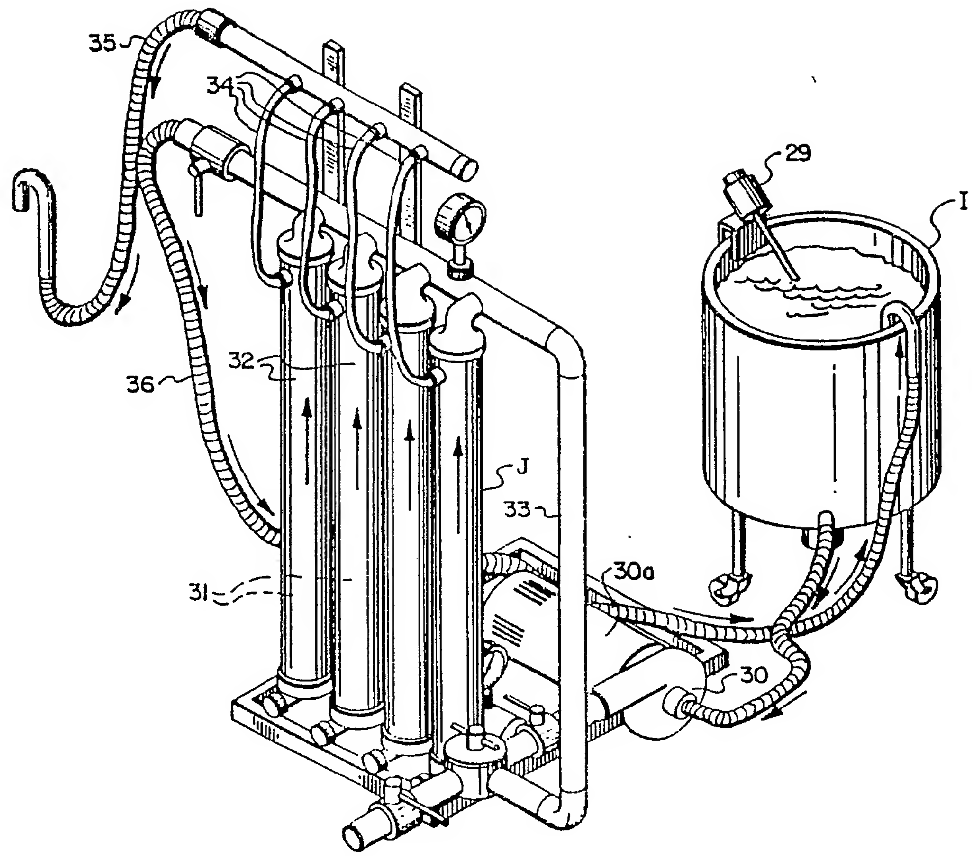


FIG. 7
DIALYZATION UNIT

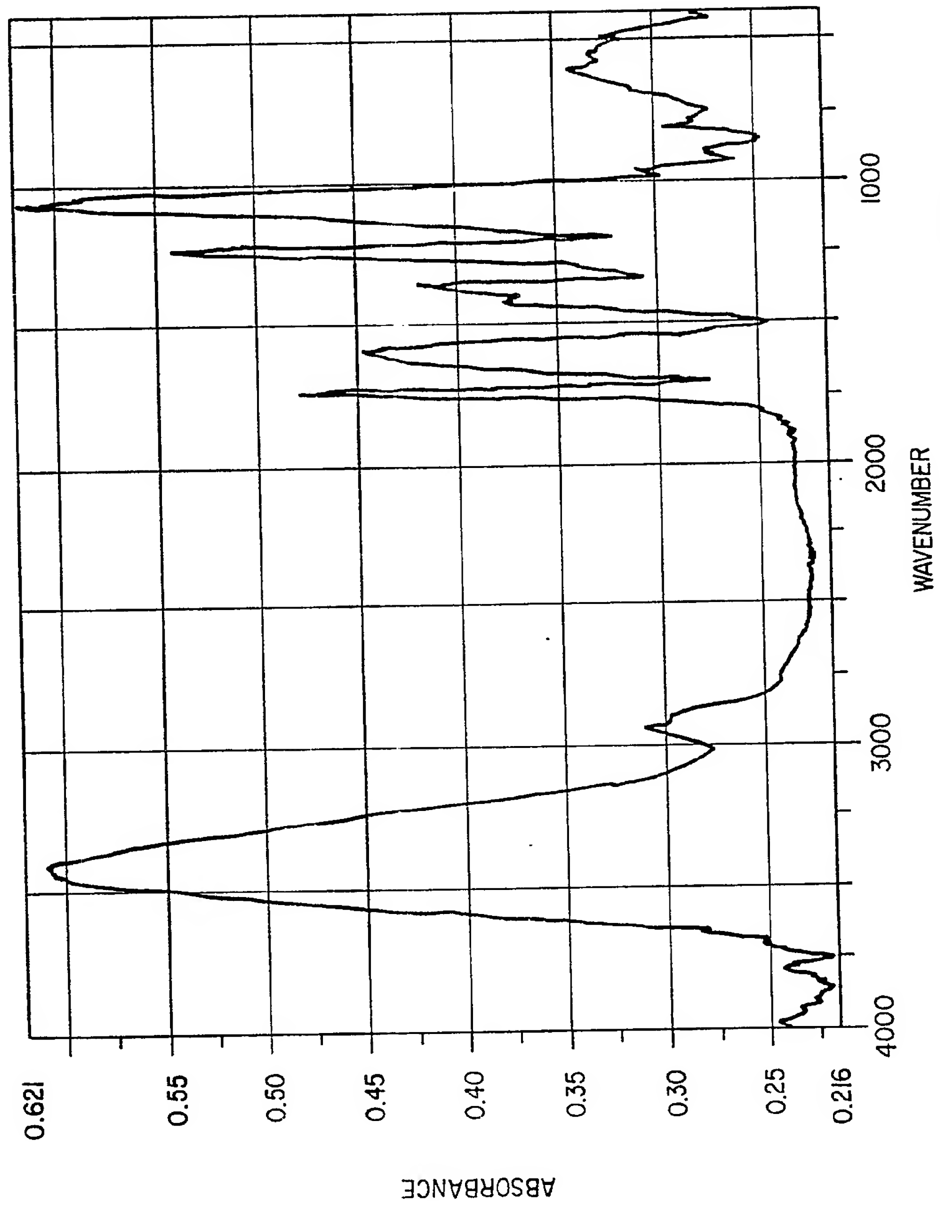
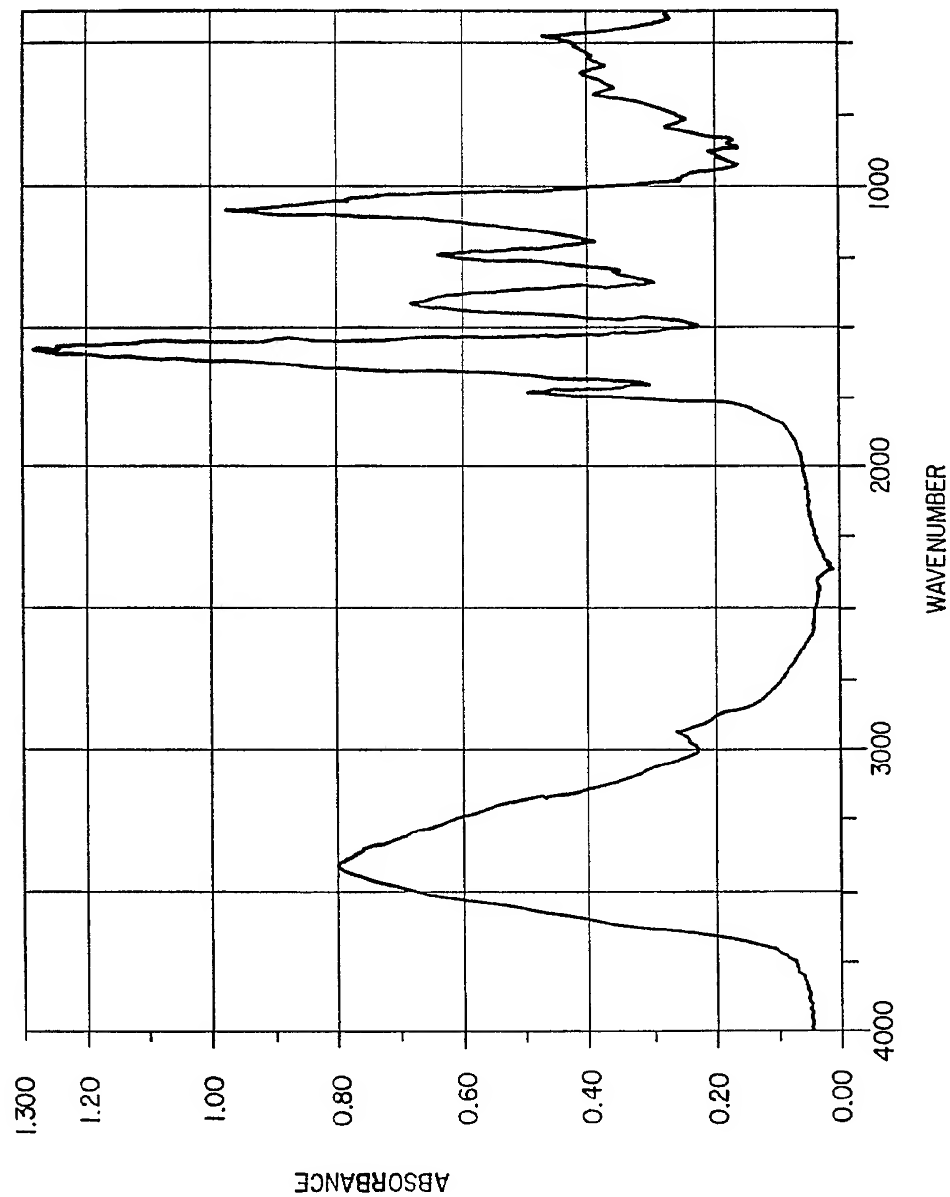

0.621
0.55
0.50
0.45
0.40
0.35
0.30
0.25
0.216
ABSORBANCE
4000
3000
2000
1000
WAVENUMBER
SCANS: 32
RES: 4 cm -1
SAMPLE: CARRISYN BATCH # I
FORM: I% IN KBR .POWDER

FIG. 8



SCANS: 30
RES: 4 cm -1

FIG. 9

SAMPLE : CARRISYN BATCH 11B
FORM: POWDER IN KBR

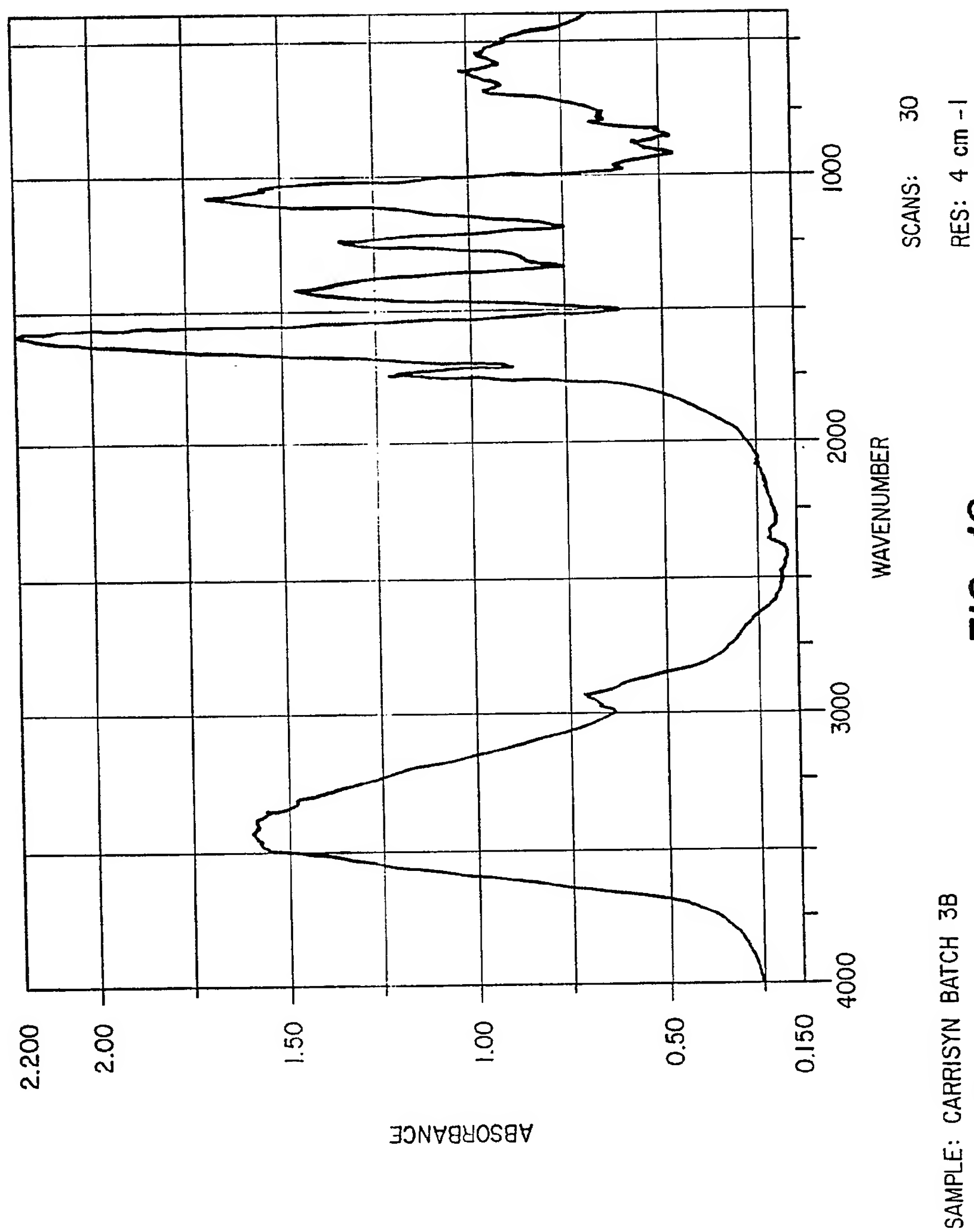

ABSORBANCE
2.200
2.00
1.50
1.00
0.50
0.150
4000
3000
2000
1000
WAVENUMBER
SAMPLE: CARRISYN BATCH 3B
FORM: POWDER IN KBR
SCANS: 30
RES: 4 cm -1

FIG. 10

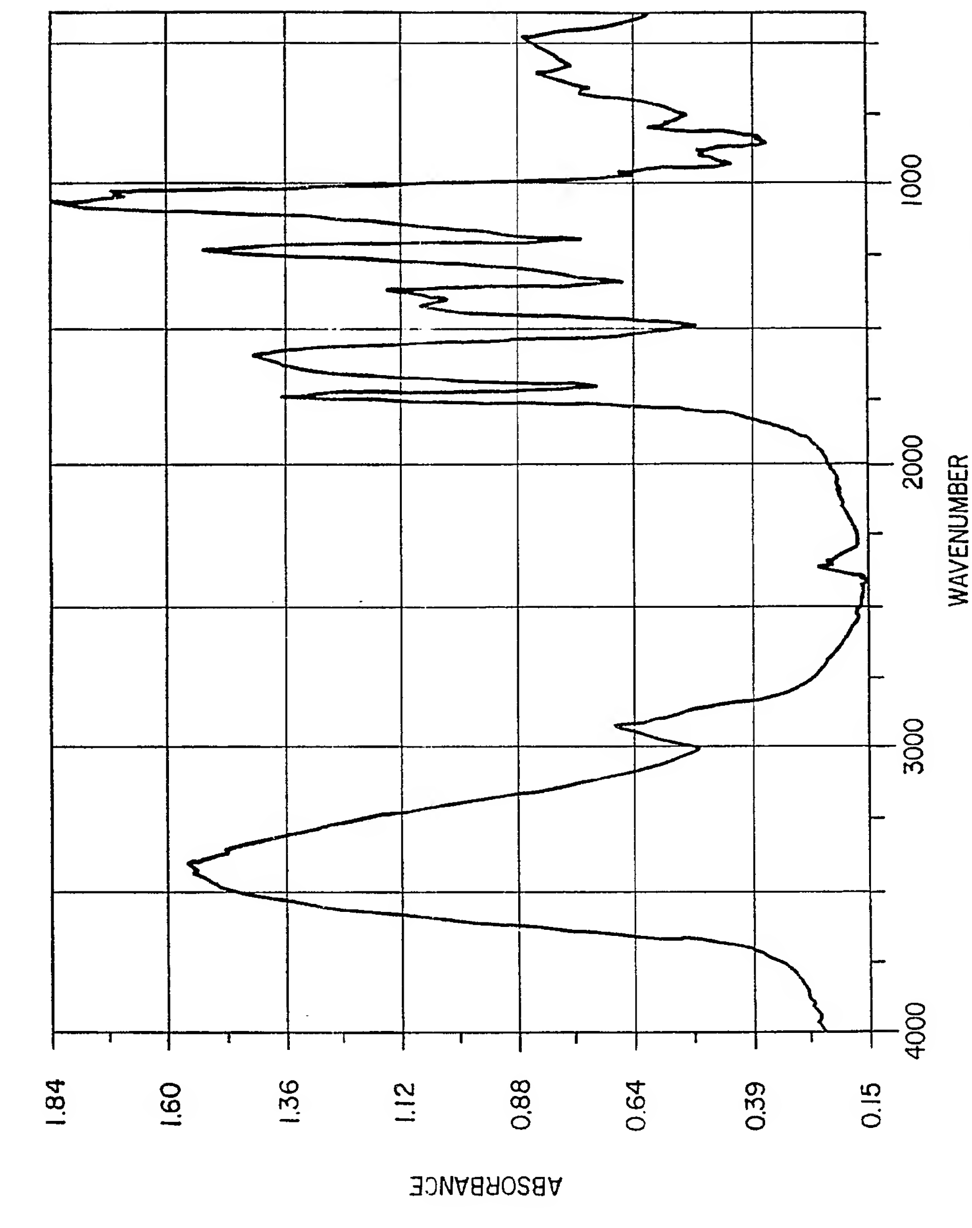
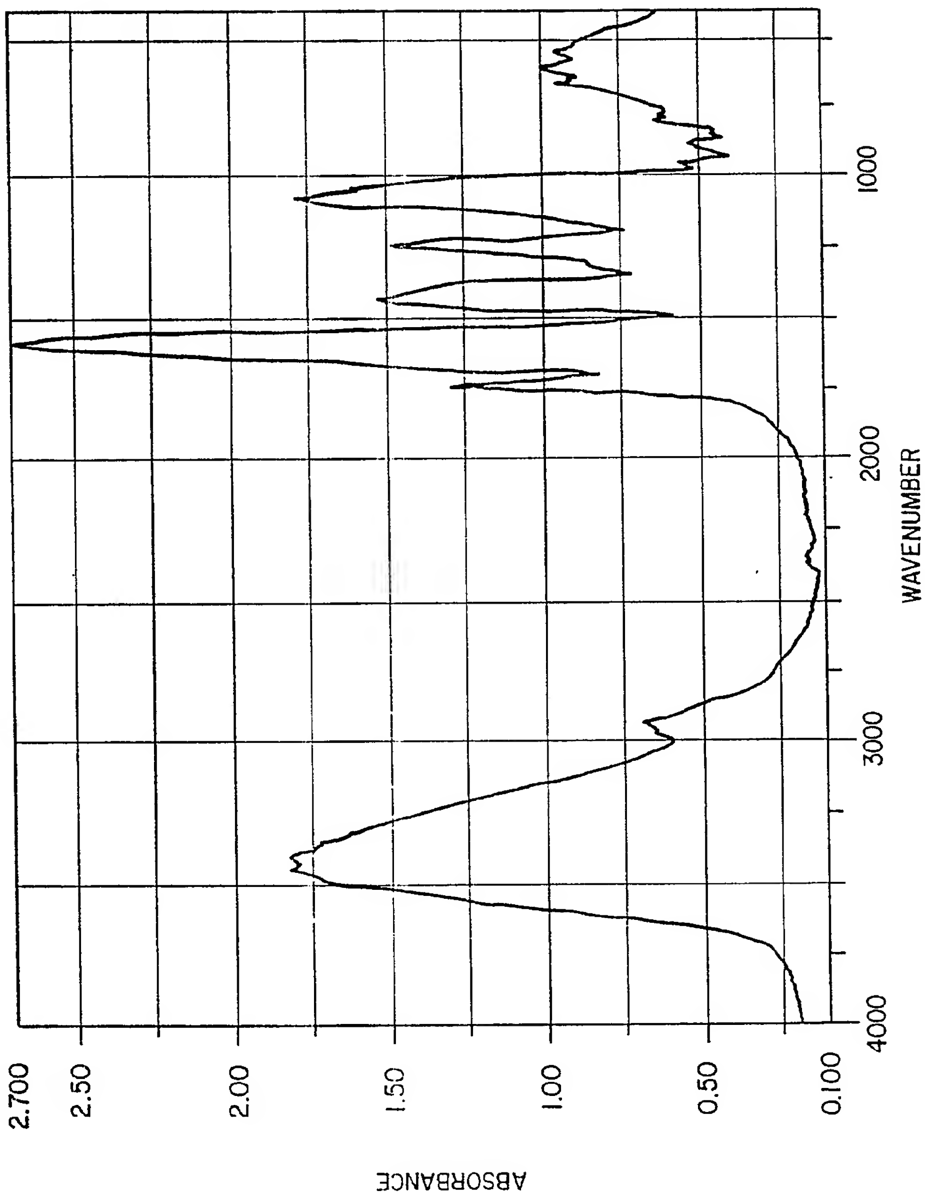

1.84
1.60
1.36
1.12
0.88
0.64
0.39
0.15
ABSORBANCE
4000
3000
2000
1000
WAVENUMBER
SCANS: 30
RES: 4 cm -1
SAMPLE: CARRISYN BATCH 5B
FORM: POWDER IN KBR

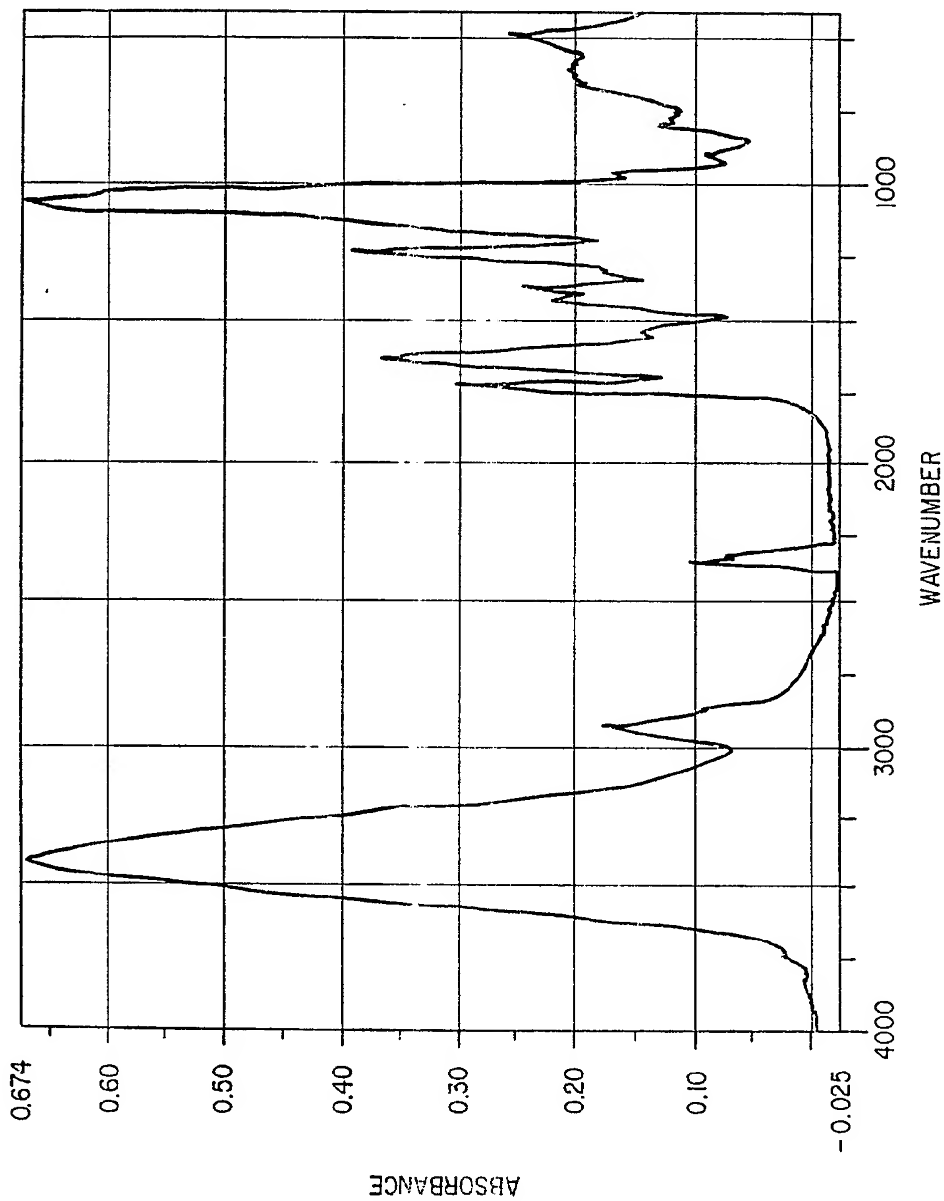
FIG. 11



SCANS: 30
RES: 4 cm -I

SAMPLE: CARRISYN BATCH 8B
FORM: POWDER IN KBR

FIG. 12



SAMPLE: CARRISYN BATCH 3B
(EXHAUSTIVELY DIALYZED - 60 HRS)

FORM: POWDER IN KBR

SCANS: 30

RES: 4 cm -1

FIG. 13

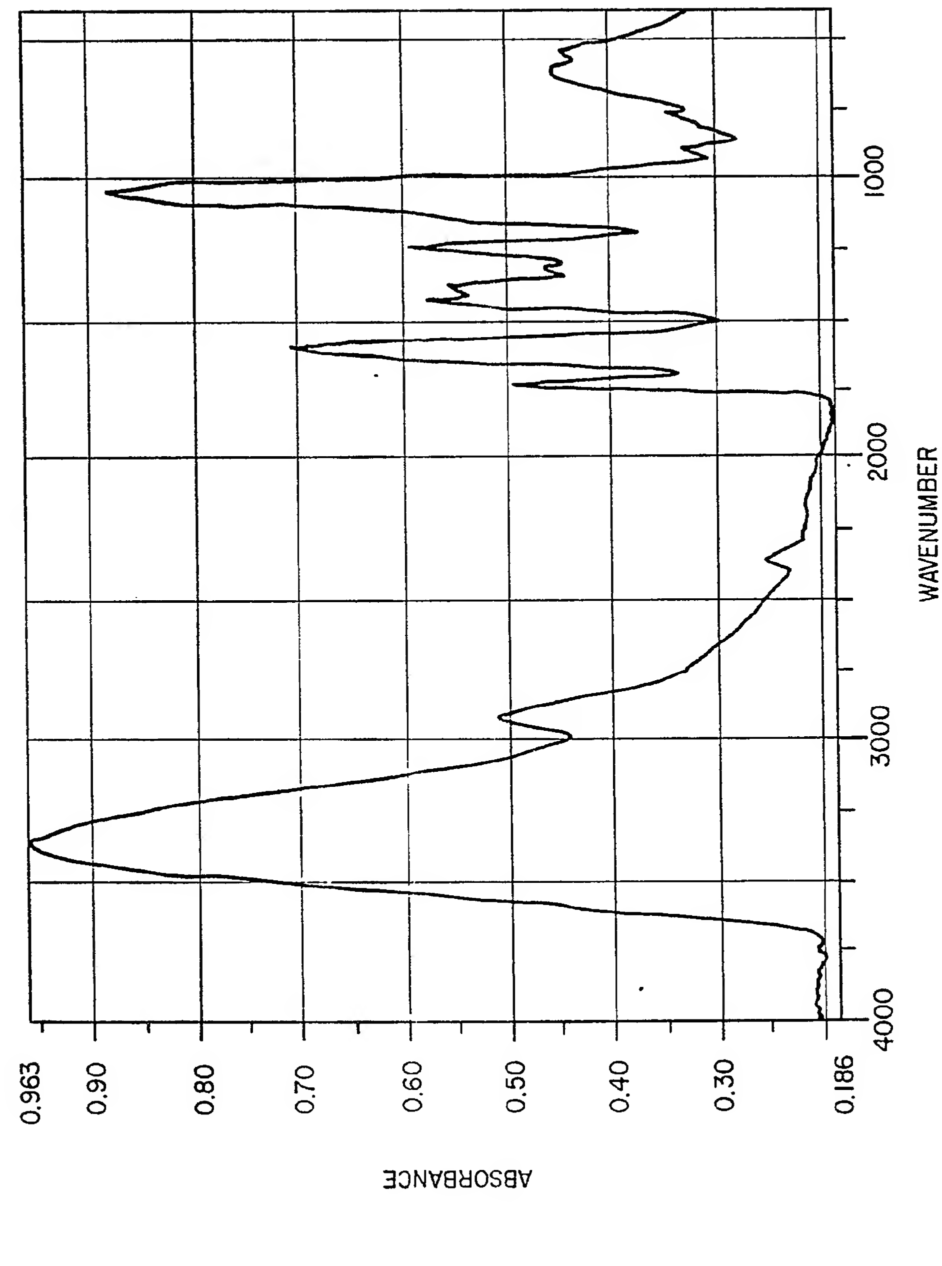

ABSORBANCE
0.963
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.186
4000
3000
2000
1000
WAVENUMBER
SCANS: 30
RES: 4 cm -1
SAMPLE: RAW ALOE GEL CAST INTO FILM
FORM: RAW ALOE VERA JUICE

FIG. 14

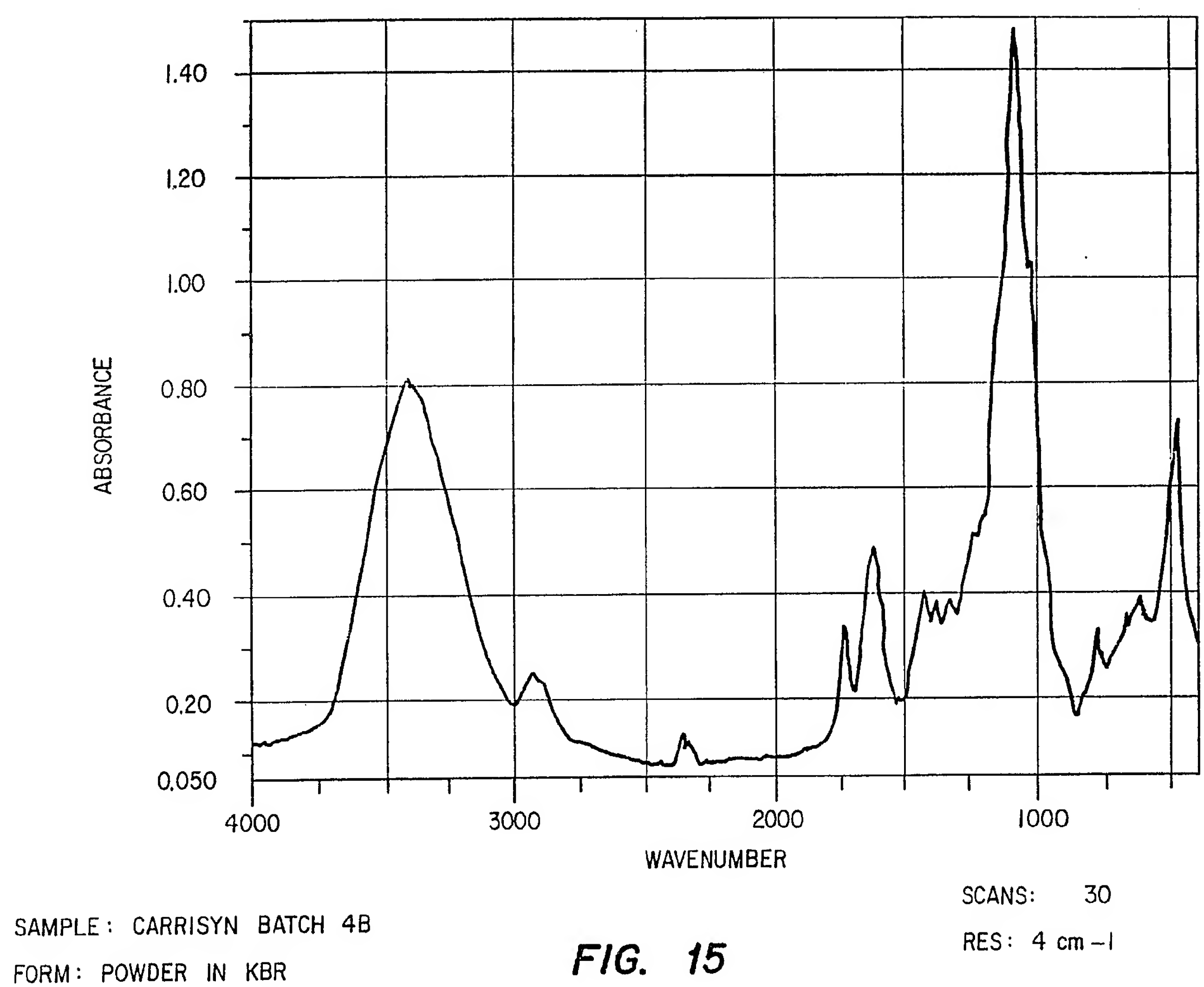
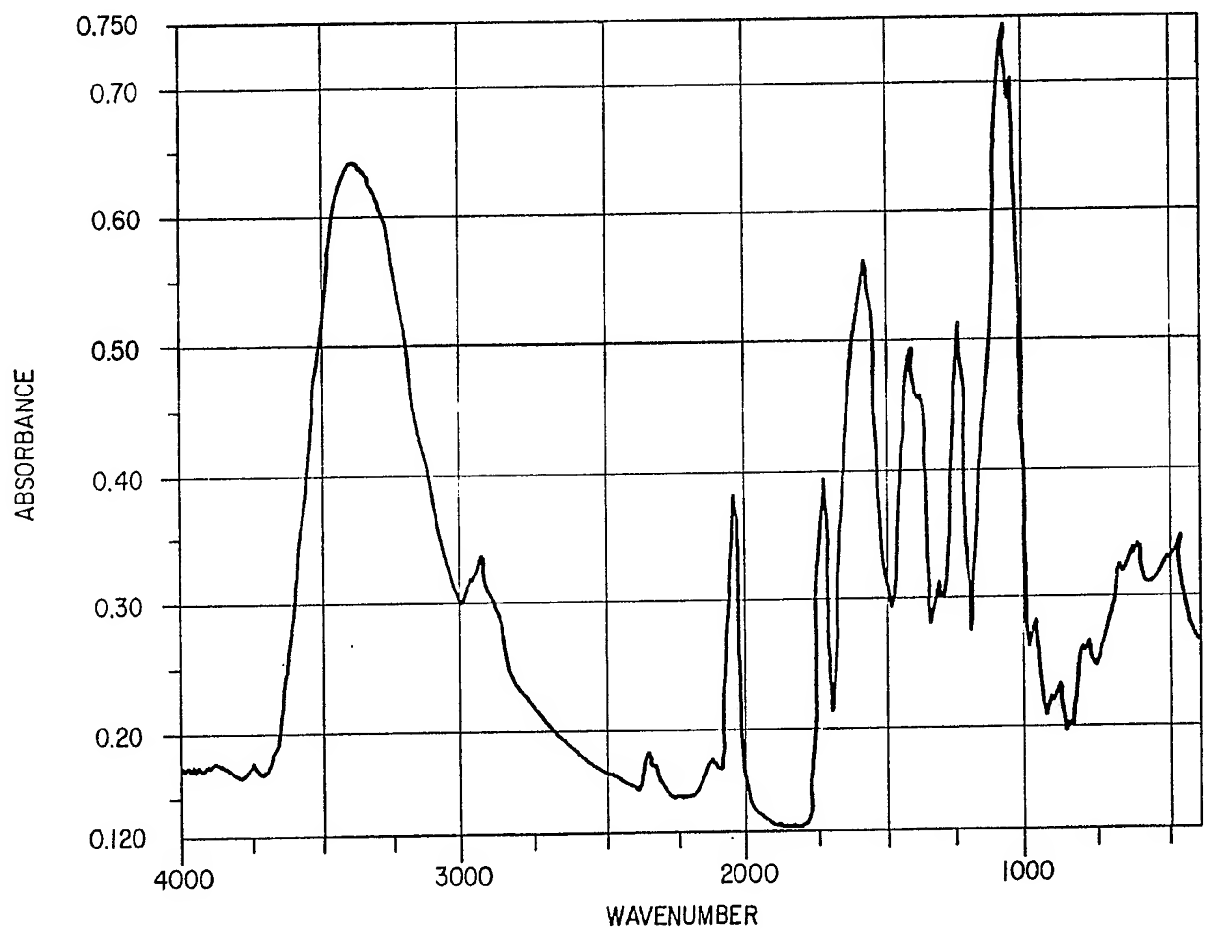

1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.050
ABSORBANCE
4000
3000
2000
1000
WAVENUMBER
SAMPLE : CARRISYN BATCH 4B
FORM : POWDER IN KBR
SCANS: 30
RES : 4 cm -1

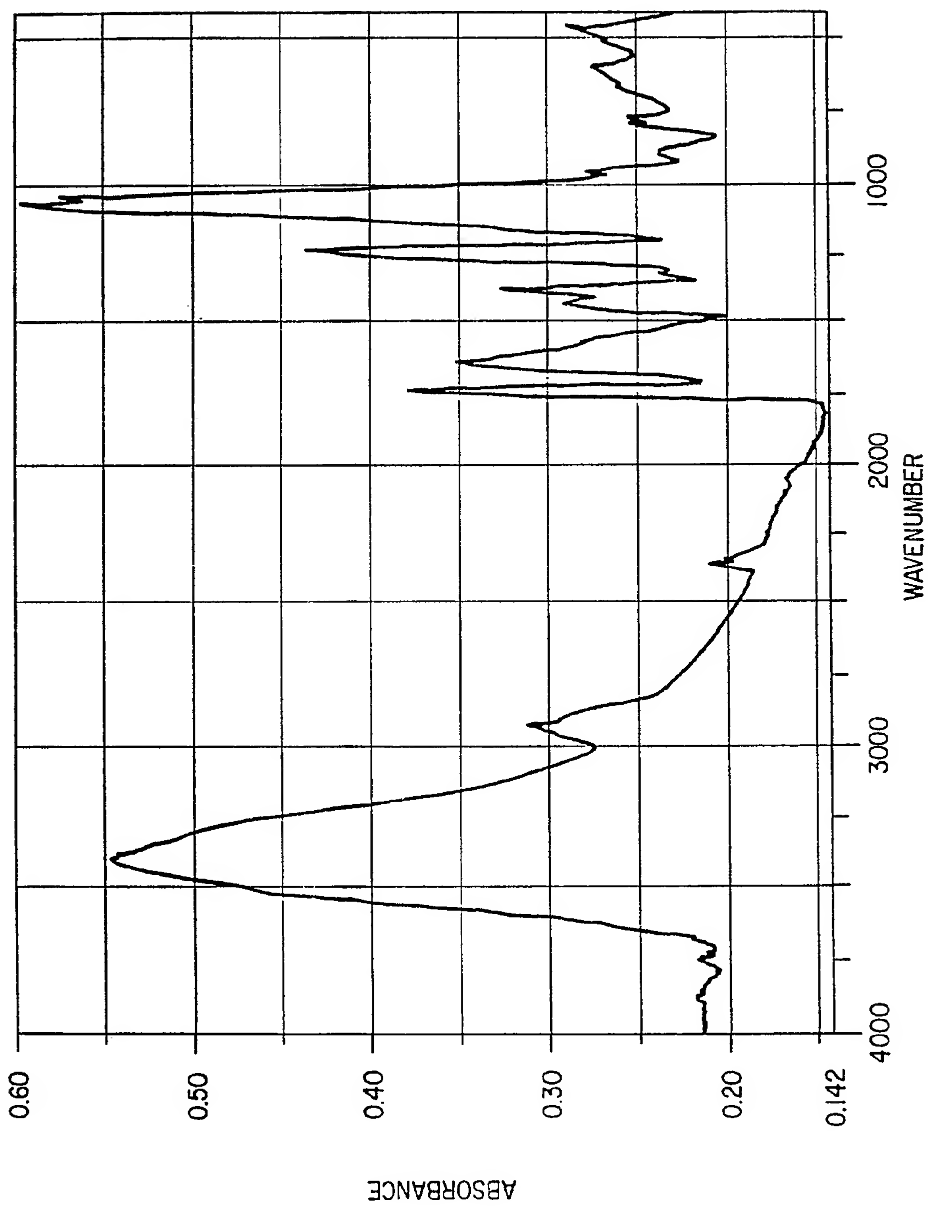
FIG. 15



SAMPLE: HILLTOP CARRISYN
(1HR BEFORE ALCOHOL)
FORM: FILM IN WATER

SCANS: 32
RES: 4 cm -1

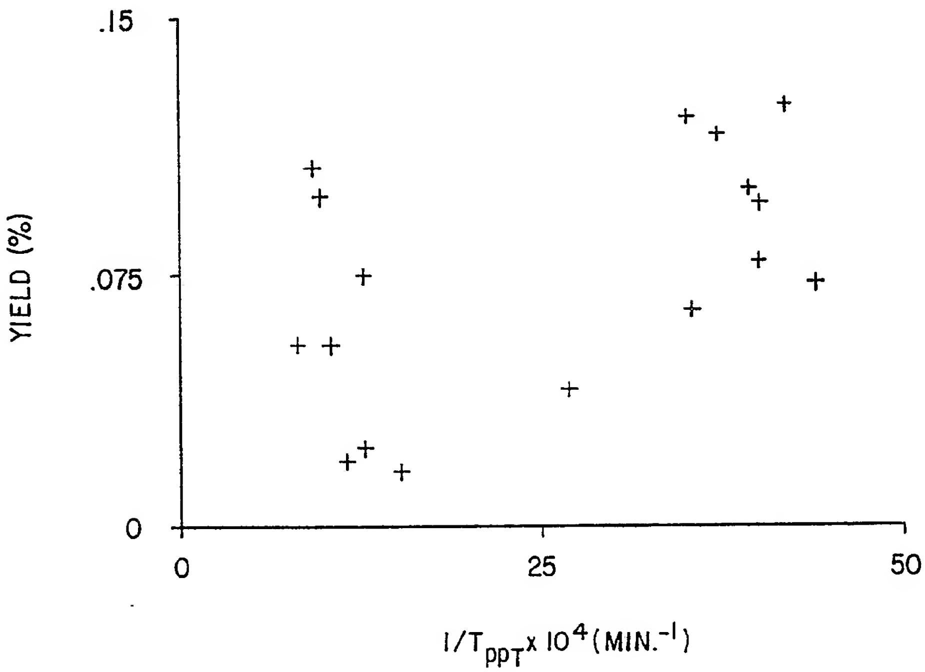
FIG. 16



SCANS: 32
RES: 4 cm -1

FIG. 17

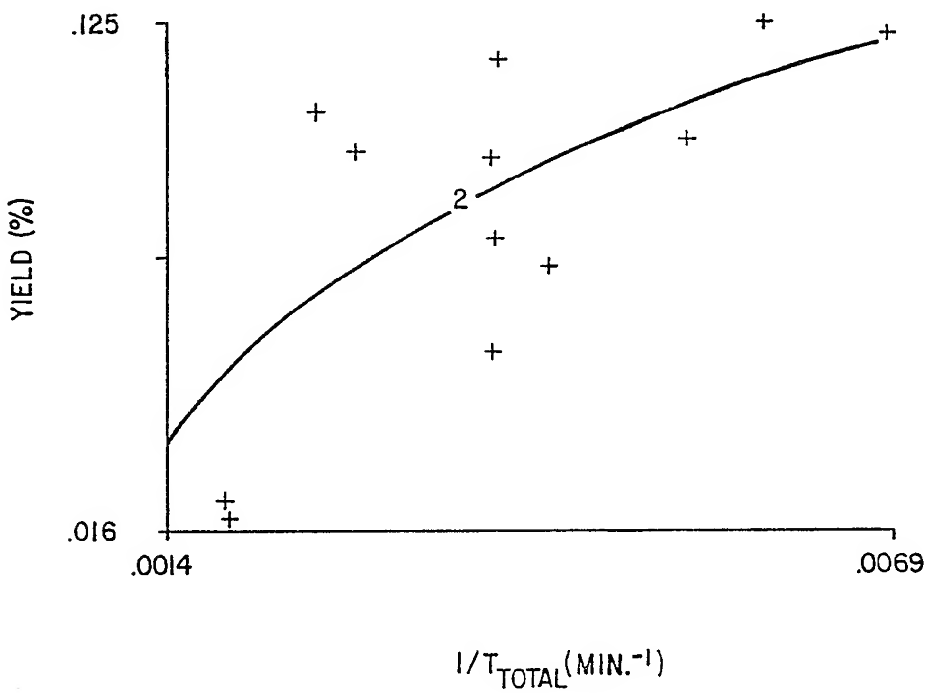
SAMPLE : CARRISYN FROM HILLTOP
(10 HRS BEFORE ALCOHOL)
FORM: FILM IN WATER



NO STATISTICALLY RELEVANT FUNCTION CAN FIT THE DATA BECAUSE OF RANDOMNESS

FIG. 18

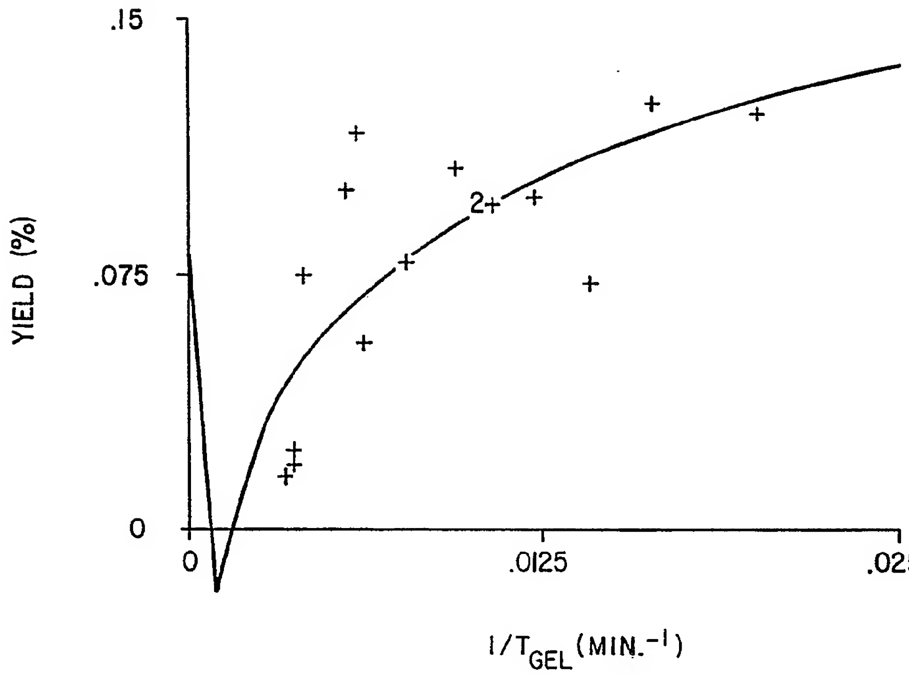
A PLOT OF CARRISYN YIELD VERSUS RECIPRICAL TIME OF GEL IN ALCOHOL (PRECIPITATION PROCESS)



BEST FIT: % YIELD = 0.053 LOG($1/T_{TOTAL}$) + 0.385
R^2 = 0.550

FIG. 19

A PLOT OF CARRISYN YIELD VERSUS RECIPRICAL TOTAL PROCESS TIME BEFORE PRECIPITATION



BEST FIT: % YIELD = 0.048 LOG ($1/T_{GEL}$) +0.314
R^2 = 0.565

FIG. 20

A PLOT OF CARRISYN YIELD VERSUS RECIPRICAL TIME OF HOMOGENIZATION AND FILTRATION

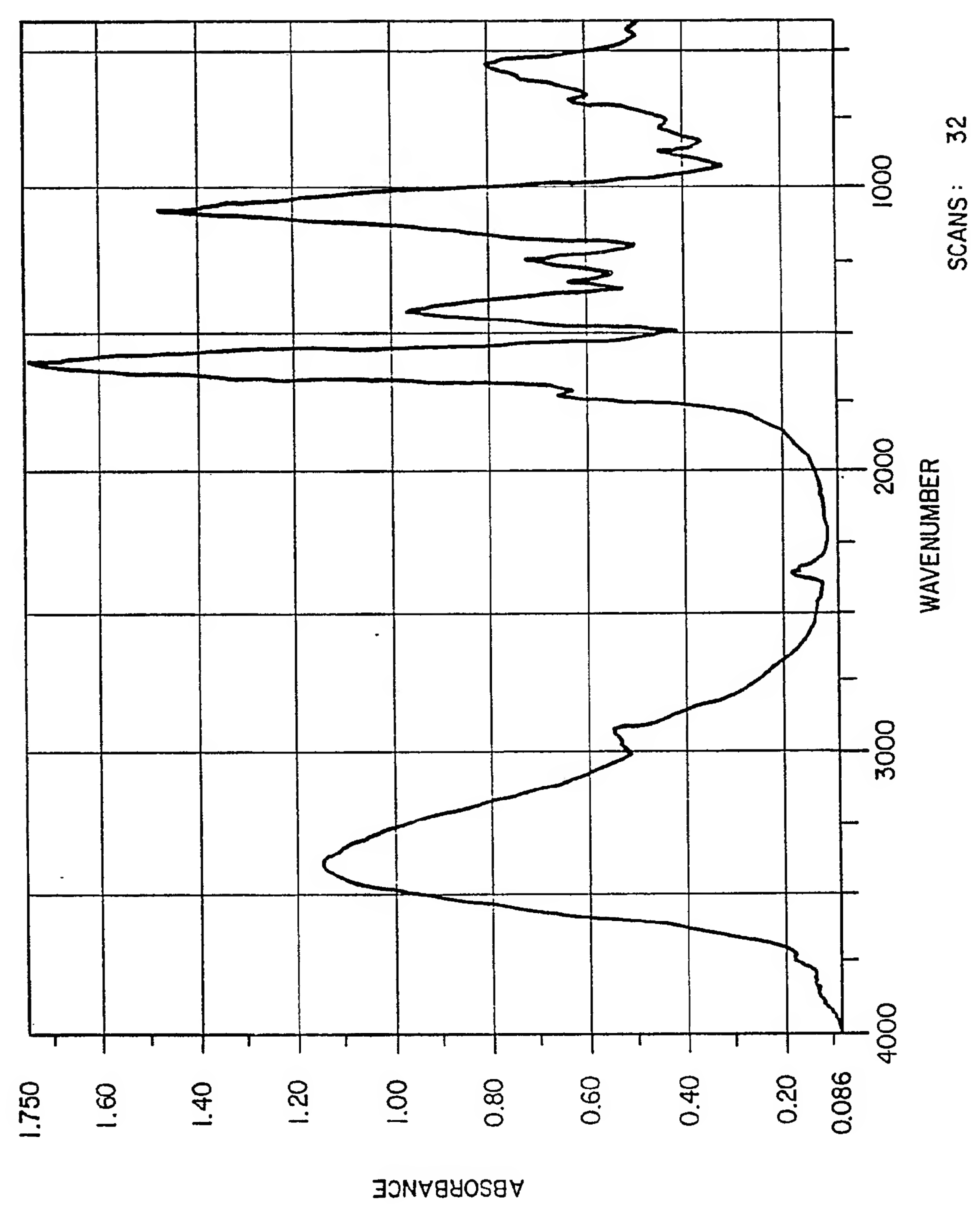
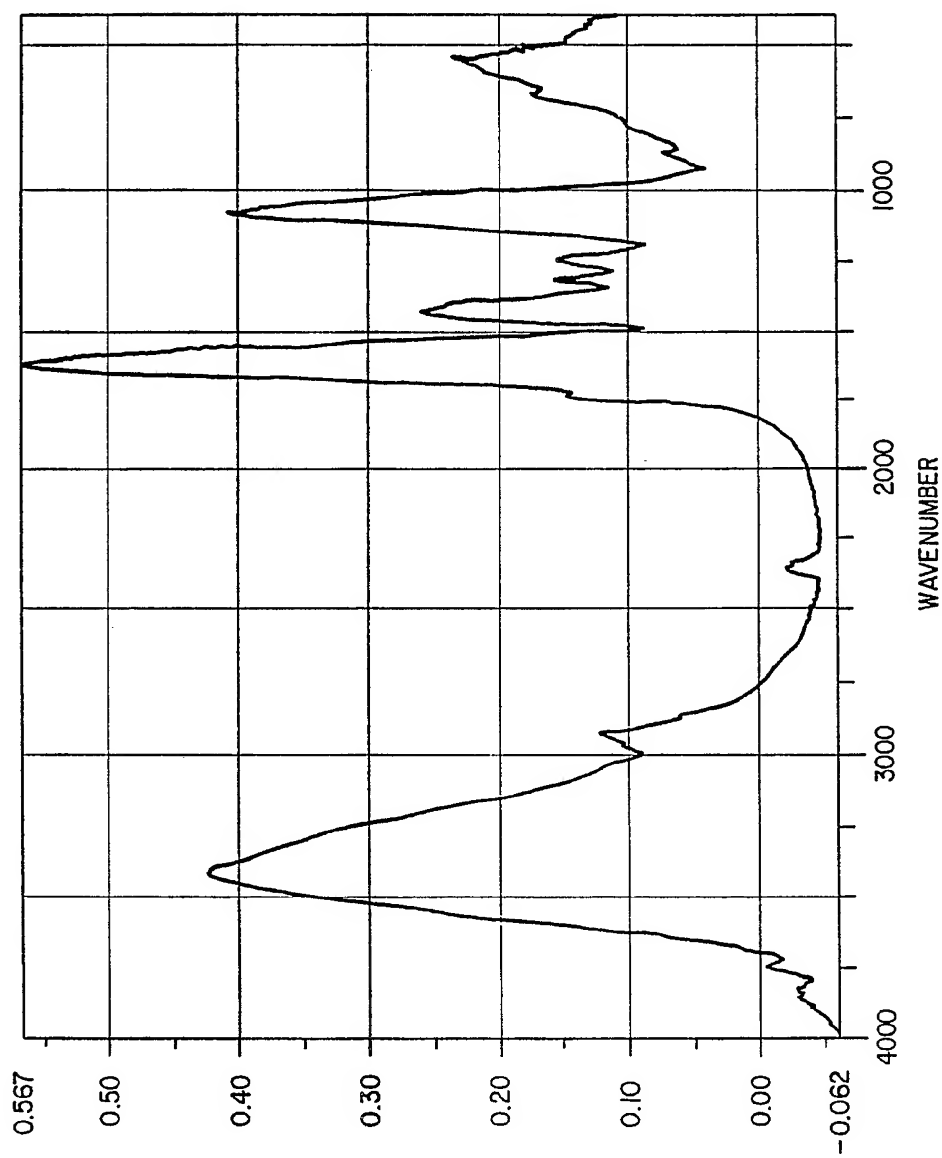

ABSORBANCE
1.750
1.60
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.086
WAVENUMBER
4000
3000
2000
1000
SCANS : 32
RES : 4 cm-1
SAMPLE : ALOE FEROX
FORM : SAMPLE IN KBR POWDER

FIG. 21



SAMPLE : ALOE AFRICANA
FORM: SAMPLE IN KBR

SCANS: 32
RES: 4 cm-1

FIG. 22

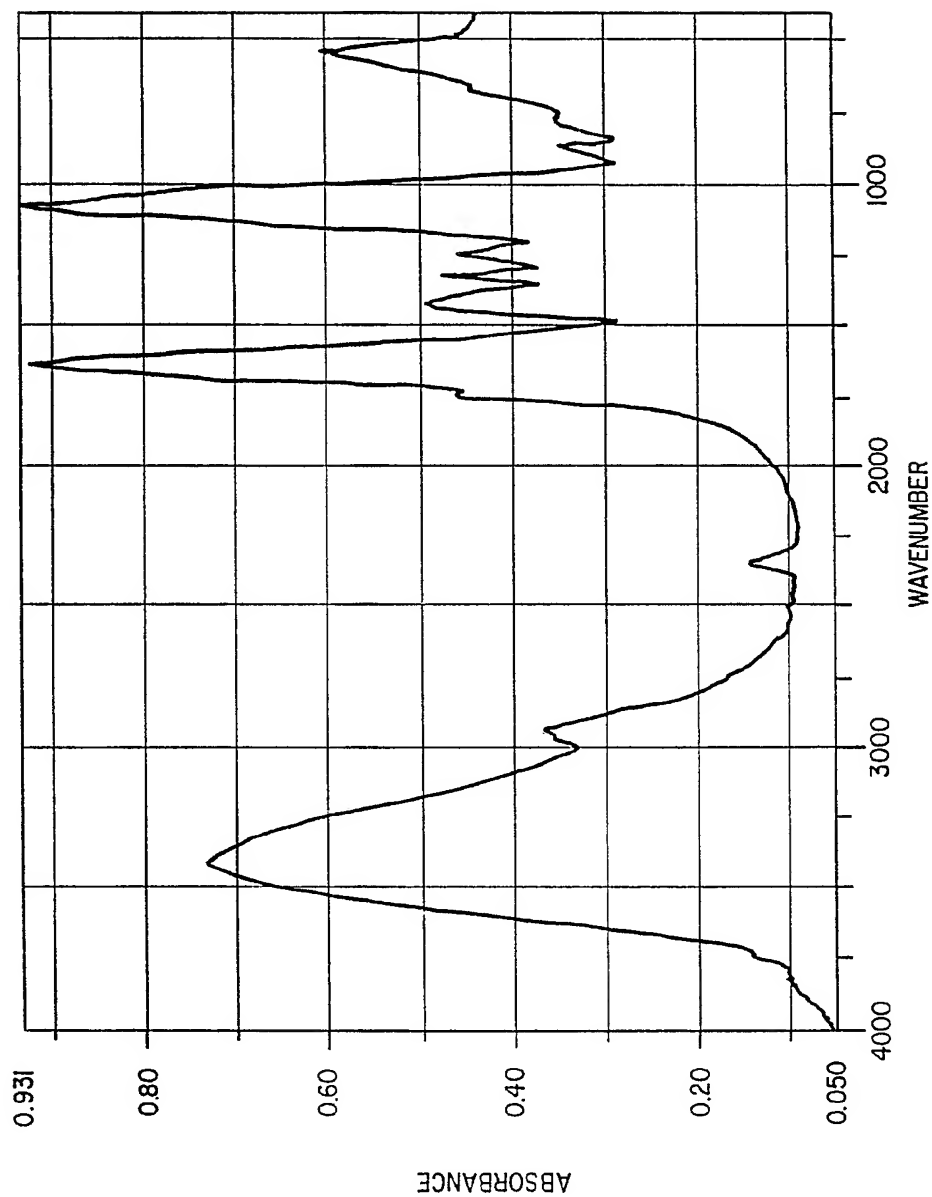
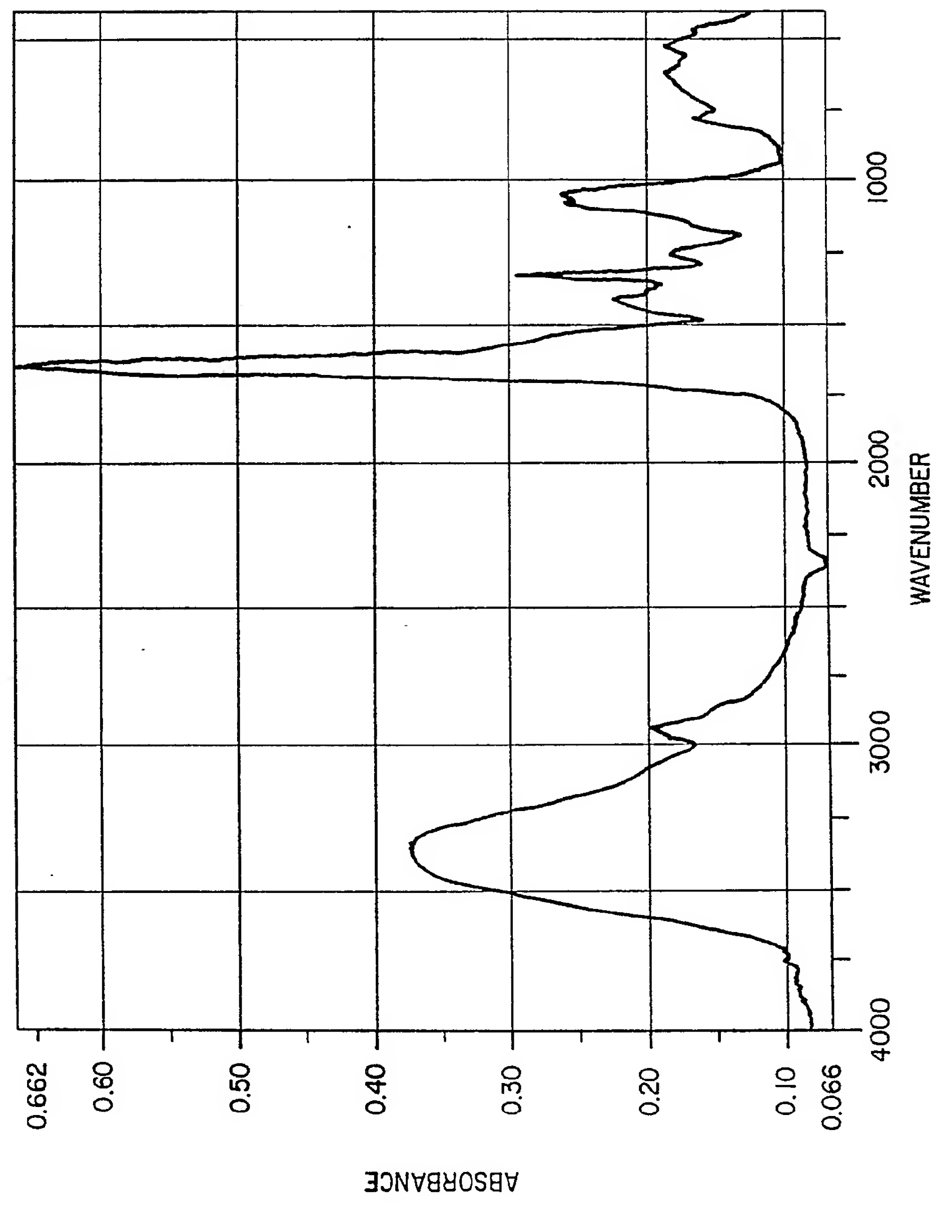


FIG. 23

SAMPLE: AFRICANA FEROX
FORM: SAMPLE IN KBR POWDER

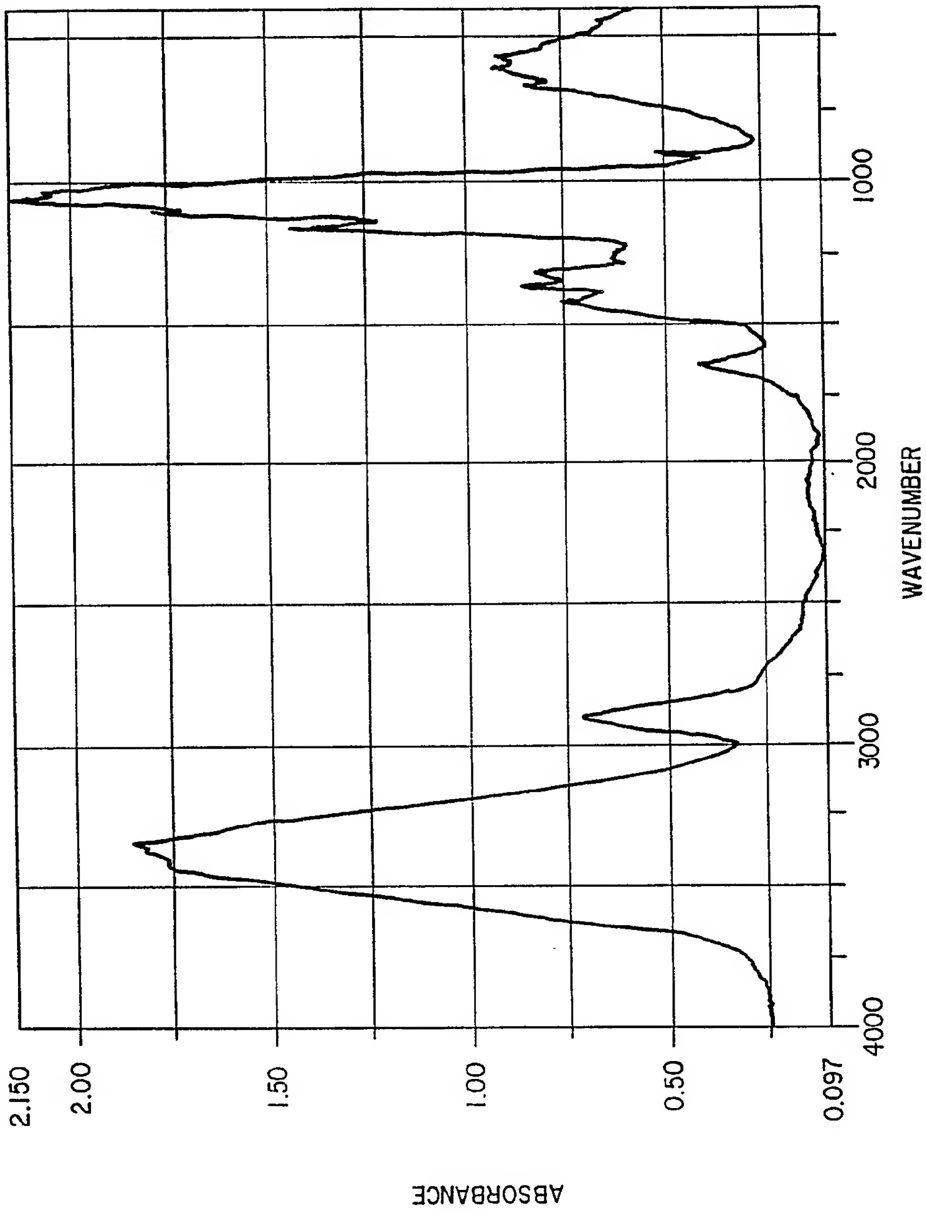
SCANS: 32
RES: 4 cm - I



SCANS: 32
RES: 4 cm-1

FIG. 24

SAMPLE: ALOE DICHOTOMA
FORM: 1% IN KBR POWDER



SCANS : 32
RES: 4 cm - I

FIG. 25

SAMPLE : CELLULOSE
FORM : POWDER

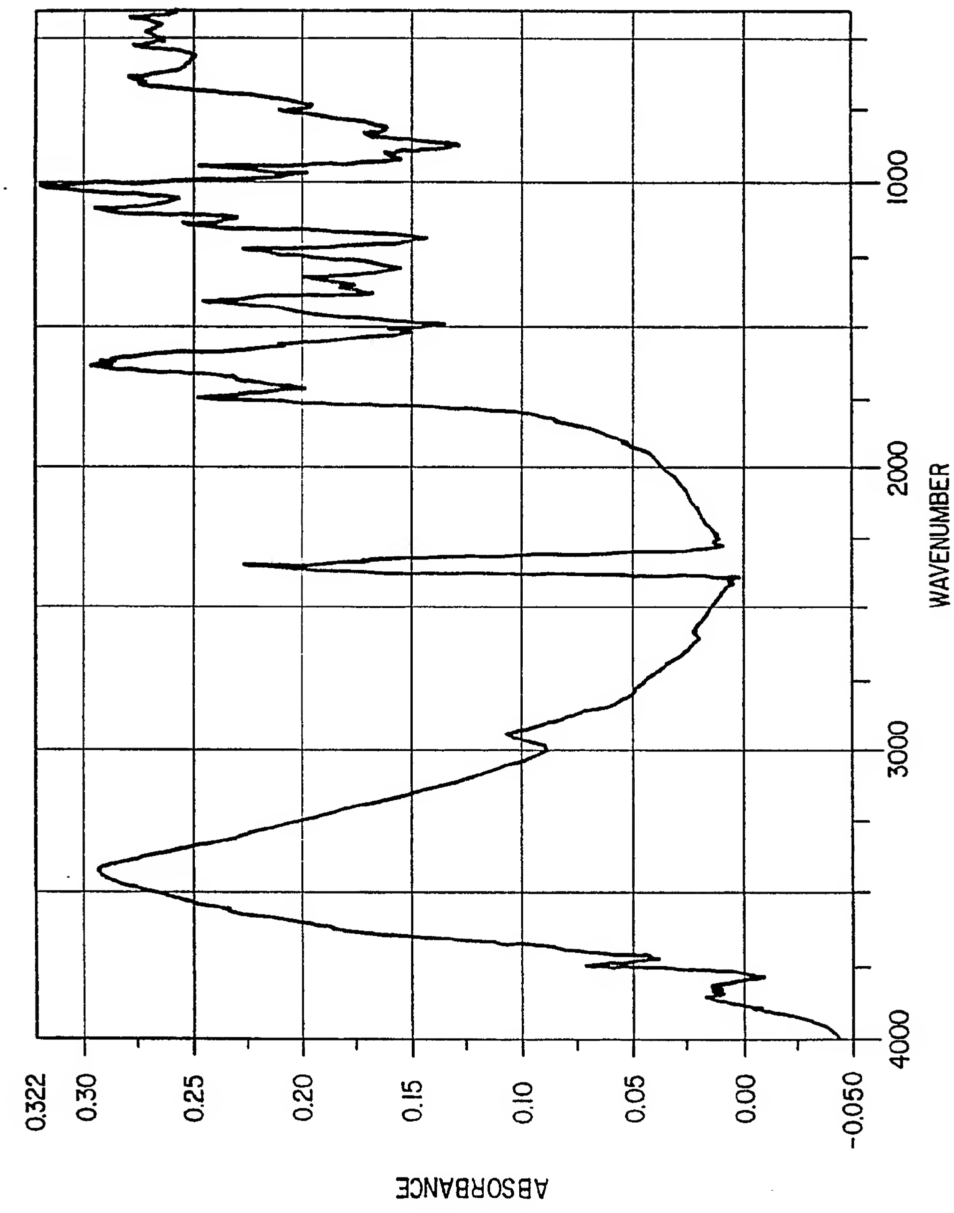

ABSORBANCE
0.322
0.30
0.25
0.20
0.15
0.10
0.05
0.00
-0.050
4000
3000
2000
1000
WAVENUMBER
SCANS : 10
RES : 4 cm -1
SAMPLE : POLYGALACTURONIC ACID
FORM : POWDER

FIG. 26

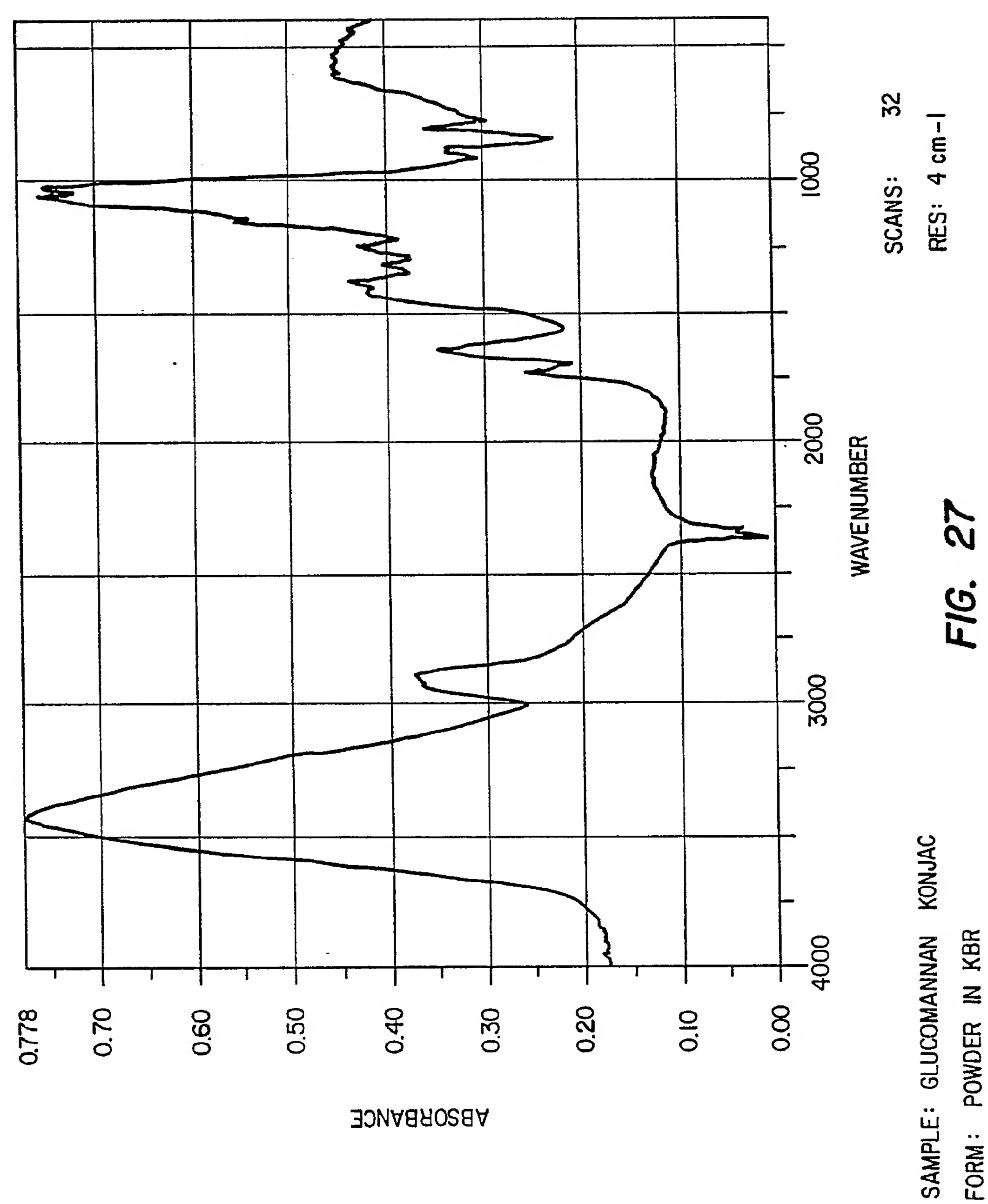

ABSORBANCE
0.778
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
4000
3000
2000
1000
WAVENUMBER
SAMPLE: GLUCOMANNAN KONJAC
FORM: POWDER IN KBR
SCANS: 32
RES: 4 cm-l

FIG. 27

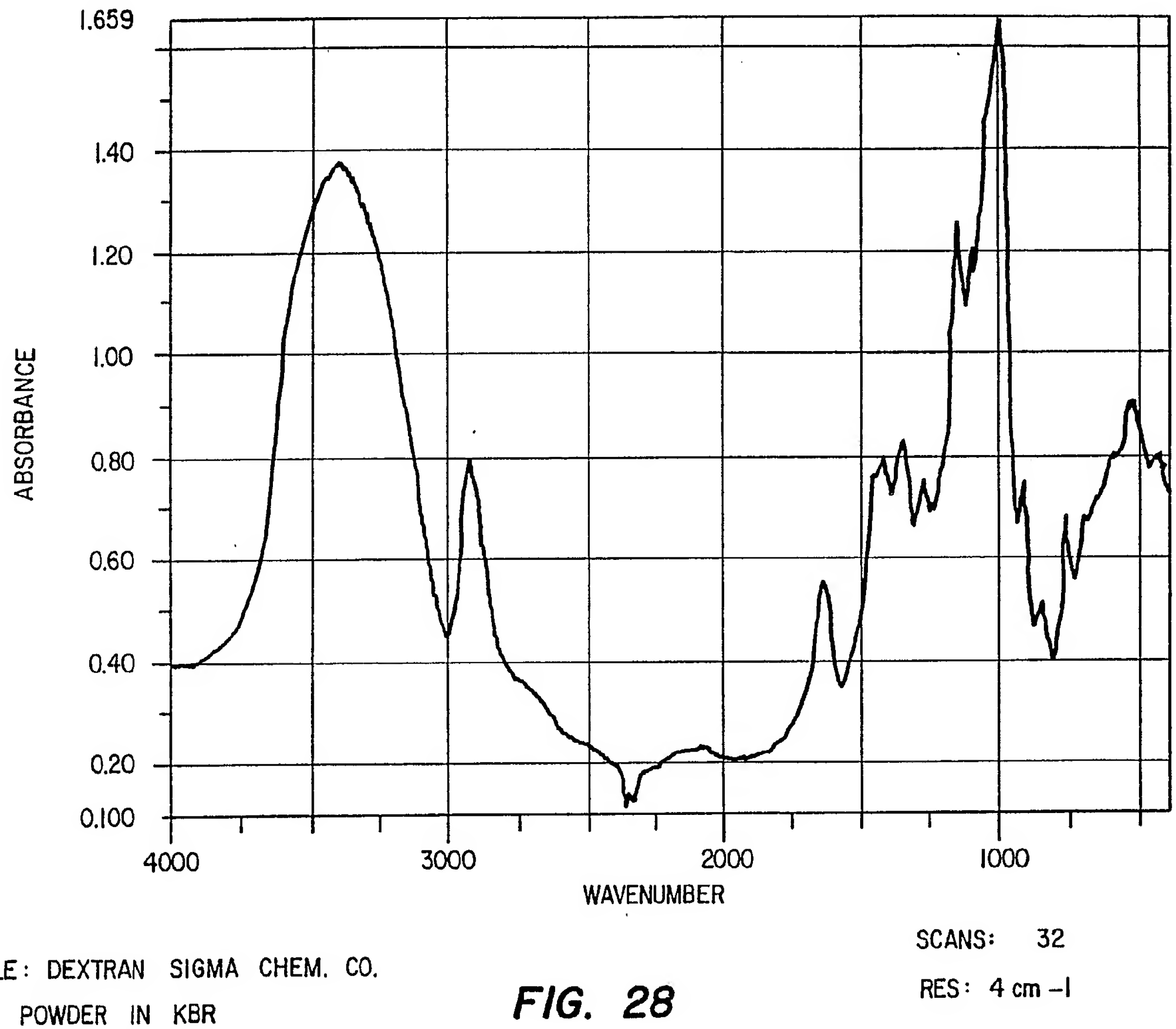
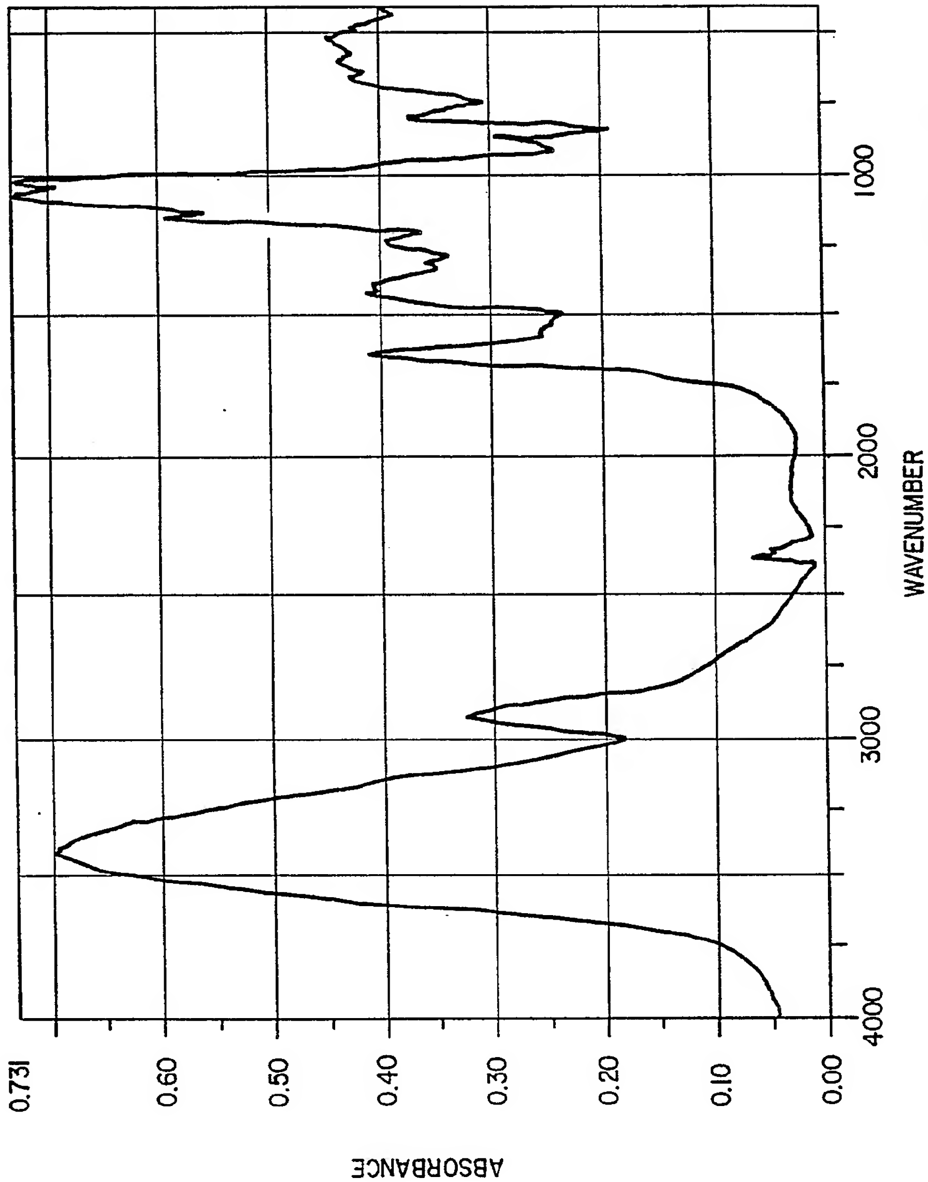

1.659
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.100
ABSORBANCE
4000
3000
2000
1000
WAVENUMBER
SAMPLE: DEXTRAN SIGMA CHEM. CO.
FORM: POWDER IN KBR
SCANS: 32
RES: 4 cm -l

FIG. 28



SCANS: 30
RES: 4 cm -1

FIG. 29

SAMPLE : GUM GUAR LOT # 101F-0279
SIGMA CHEM. CO.
FORM : POWDER IN KBR

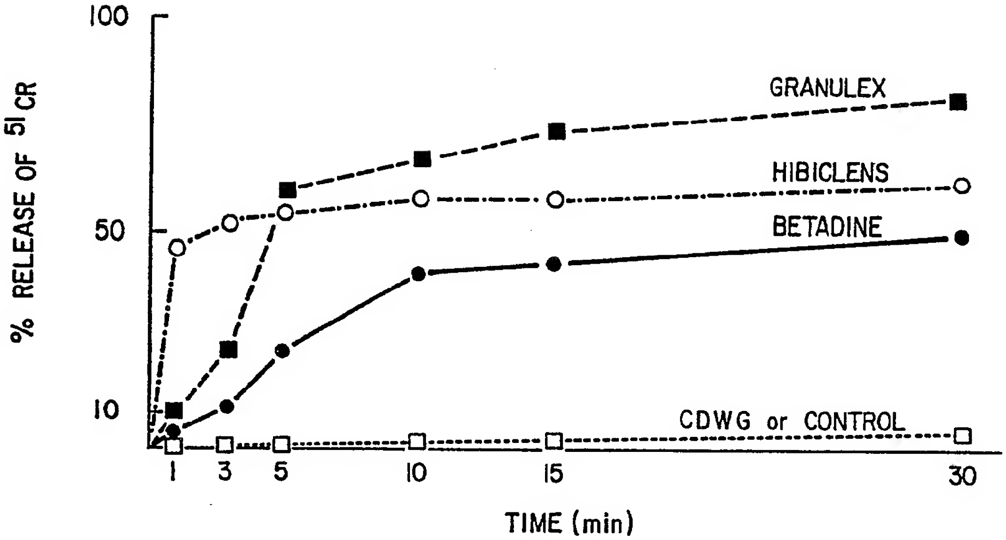

100
50
10
% RELEASE OF 51CR
GRANULEX
HIBICLENS
BETADINE
CDWG or CONTROL
1
3
5
10
15
30
TIME (min)

FIG. 30

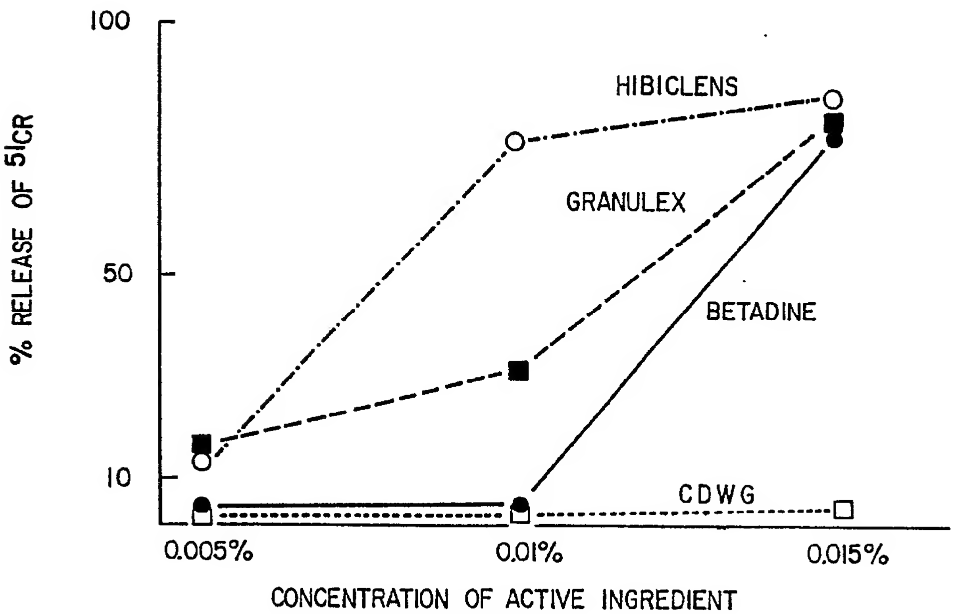

100
50
10
% RELEASE OF 51CR
HIBICLENS
GRANULEX
BETADINE
CDWG
0.005%
0.01%
0.015%
CONCENTRATION OF ACTIVE INGREDIENT

FIG. 31

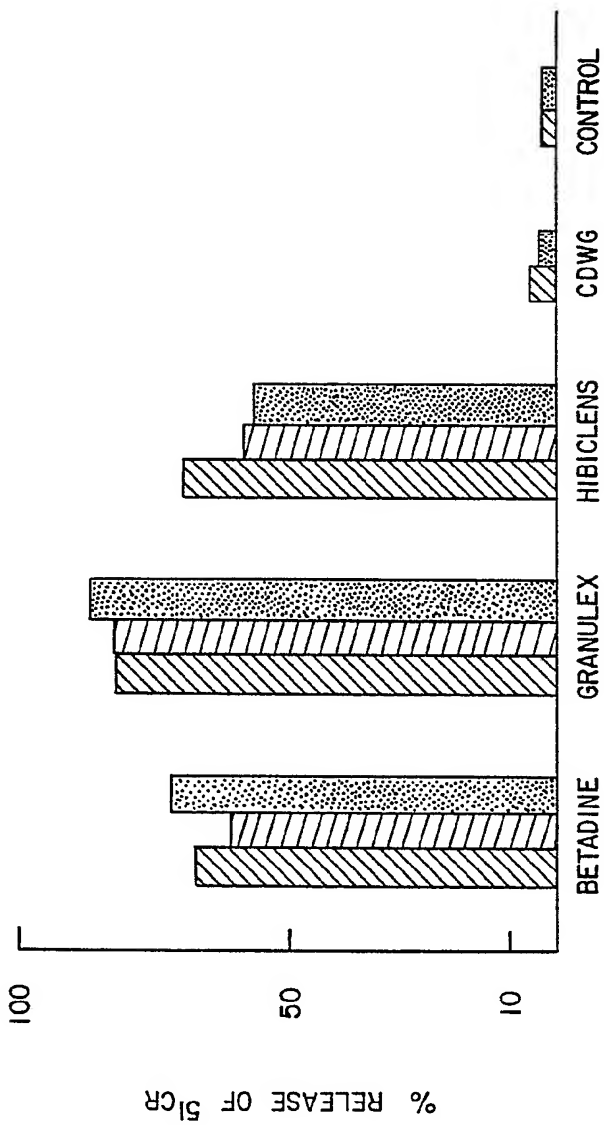

% RELEASE OF 51CR
100
50
10
BETADINE
GRANULEX
HIBICLENS
CDWG
CONTROL

FIG. 32

European Patent Office

EUROPEAN SEARCH REPORT

Application Number

EP 88 12 1318

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.4)
A	SEIFEN-ÖLE FETTE-WACHSE, vol. 105, no. 17, October 1979, pages 499-502, Augsburg, DE; P. HOFFENBERG: "Aloe Vera. Eine alte Heilpflanze - neu für die Kosmetik"		A 61 K 35/78 A 61 K 7/48 A 61 K 31/715 C 08 B 37/00
A	US-A-3 360 511 (A. FARKAS)		
A	FR-M- 3 068 (A. FARKAS)		
A	US-A-4 446 131 (R.G. MAUGHAN)		
A	US-A-4 465 629 (R.G. MAUGHAN)		
A,D	US-A-3 892 853 (H.H. COBBLE)		
A,D	US-A-4 178 372 (B.C. COATS)		
			TECHNICAL FIELDS SEARCHED (Int. Cl.4)
			A 61 K

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	17-04-1989	REMPP G.L.E.

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
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